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By

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Declaration

The experimental work presented in this thesis was carried out at the University of KwaZulu-Natal, Pietermaritzburg and at Owen Sitole College of Agriculture (OSCA), Empangeni. The research was carried out according to UKZN animal ethics policy (Reference: 086/11/Animal). The work was supervised by Professor Ignatius V. Nsahlai and Co-supervised by Professor Peter F. Scogings.

This to declare that this thesis represent original work done by myself and have not been submitted in any previous application for a degree. All sources of information are shown in the text and listed in the reference and all assistance by others has been duly acknowledged.

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I declare that the above statement is correct.

Supervisor Prof. Ignatius V. Nsahlai

Signed……. Date…….

Co-supervisor Prof. Peter F. Scogings

Signed…… Date……..

Dedication

To my father ***Abdelrahim Dafaalla****, my mother* ***Maria Elawad****, my father in law* ***Alhag Ali****, my mother in law* ***Elbakira Ibrahim****, my beloved wife,* ***Mawahib Alhag****, my treasured daughter* ***Aseel*** *and precious son* ***Mohamed.***

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Nasreldin Abdelrahim Dafaalla Basha

General Abstract

Diet selection and its variation due to difference in forages nutritive value are central processes in plant-herbivore interactions. An incorporating of these parameters may allow predicting diet selection patterns which are a basic underpin for sustainable vegetation management and profitable animal production. To achieve these issues many studies were done two of which were run to study the browse-browser interactions in a sub-humid subtropical savannah, a herd of goats was used as a model browser in natural pasture. Other studies evaluated the nutritive value of five plant species which were the most selected by goats.

The objectives of this study were to (i) determine the seasonal patterns of diet selection of goats on woody species and their nutritive value and (ii) to predict the diet selection. The secondary objectivrs were to (i) investigate how plant characteristics such as plant morphology (spinescence, and broad vs. fine leaves), phenology (evergreen vs. deciduous species) and plant chemistry (nitrogen, neutral detergent fibre, acid detergent fibre, acid detergent lignin, condensed tannin, cellulose and hemicellulose) are relate to the diet selection patterns and to (ii) evaluate the nutritive value of the most plant species selected by goats during the different seasons.

Two experiments were conducted at Owen Sitole College of Agriculture, KwaZulu-Natal, to determine diet selection of goats grazing/browsing in Zululand Thornveld. The first experiment was done in the early wet season (November 2007) and late wet season (March/April 2008) while the second experiment was conducted in the dry season (June/July 2008), early wet season (November/December 2008) and late wet season (February/March 2009). The relative abundance of browse species in the veld was determined and used to estimate the selection index. In the first part, diet selection was estimated using (i) direct observation of animals (account bouts) by observing every one minute two goats while they were foraging with others for 3 hours in the morning and 1.5 hours in the afternoon on alternate days for a total of six days and (ii) an indirect plant-based method by observing at regular intervals 40 marked branches on ten plants of each browse species every two days for a total of six days. The browse species observed were: *Gymnosporia senegalensis*, *Acacia nilotica*, *Acacia karroo* (*Acacia natalitia*), *Scutia*

myrtina and *Dichrostachys cinerea*. In both seasons, the three most selected species according to the plant-based observations were *S. myrtina*, *A. karroo* and *D. cinerea*. Consistently, *A. nilotica* experienced moderate defoliation and *G. senegalensis* the least. However, on the basis of the selection index, the species followed the order: *A. nilotica* > *D. cinerea* > *A. karroo* > *S. myrtina* > *G. senegalensis* in the early wet season and *A. nilotica* > *S. myrtina* > *D. cinerea* > *A. karroo* > *G. senegalensis* in the late wet season. Both methods did not rank species in the same order. The selection index was negatively ($P<0.05$) correlated to neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). In the second part, observations were taken on feeding time of two goats, randomly selected per day for 7-8 days. Observations were made during active foraging periods for 2 hrs in the morning and 1.5 hrs in the afternoon. The duration of each feeding bout and the species of woody plant from which bites were cropped at each feeding station were recorded. Season and plant species affected diet selection and preference. The five main species selected (utilized) by goats in decreasing order were *S. myrtina*, *A. nilotica*, *D. cinerea*, *Acacia natalitia* and *Chromolaena odorata*. *Scutia myrtina* was the most preferred (highest utilisation relative to availability) in the dry season while *D. cinerea* comprised the greatest proportion in the early and late wet seasons. *Scutia myrtina* was most preferred in the dry and early wet seasons while *A. nilotica* was most preferred in the late wet season. Spinescent species were generally selected more than non-spinescent species in all seasons, while fine-leaf and deciduous species were selected more than broad-leaf and evergreen ones in the wet seasons. However, preference for broad-leaf and evergreen species increased in the early wet season. Although plant chemistry varied across seasons, it did not explain the preference of goats for various plant species in this study. Instead, effects of chemistry were species- specific.

The nutritive value of the five main species selected by goats was evaluated by chemical composition, *in vitro* gas production, *in vitro* degradability and *in sacco* degradability.

Two experiments were conducted for the *in vitro* studies, the first experiment was to test the effect of season and species, while the second experiment tested the biological effect of tannins using polyethylene glycol 4000 (PEG). The PEG treatment was applied to samples of the early and late wet seasons only. The parameters were maximum gas production (GP), degradation rate (C), lag time (lt), maximum rate of GP at the point of inflection (μ), half time to the maximum gas volume($T_{1/2}$), gas produced from fermentation of soluble

and slowly degradable fractions (A and B, respectively), their degradation rates (c1 and c2, respectively), apparent (ApDeg) and true degradability (TrDeg), microbial yield (MY), partitioning factor (PF), degradation efficiency factor (DEF) and short chain fatty acids (SCFA). There were wide variations among seasons and species in crude protein (CP), NDF, ADF and condensed tannins. Season and species affected kinetics of gas production, GP, TrDeg, MY, PF and SCFA. *Chromolaena odorata* had the highest CP (185.8-226.4 g kg^{-1}), GP (87.3-104.1 ml g^{-1} DM), gas produce from soluble (47.6-50.9 ml g^{-1} DM) and insoluble (39.8-50.9 ml g^{-1} DM) fractions during the three seasons compared to other browse species. The TrDeg ranged from 634 to 856 g kg^{-1} DM. The total SCFA varied between seasons and among species. The addition of PEG decreased TrDeg and PF, and increased GP and total SCFA. The GP, its degradation rate (C) and gas from the soluble fraction were positively correlated to CP without and with PEG. Gas from the soluble fraction was negatively correlated to NDF, ADL and CT; and GP to CT without PEG.

In the *in sacco* study, the parameters of dry matter (DM) and nitrogen (N) degradability were soluble (*a*) and slowly (*b*) degradable degradability, the degradation rate (*c*), potential degradable (*PD*), effective degradability (*ED*) and lag time (*lt*). Season affected *PD* of DM degradability and *ED* of both DM and CP. Browse species and its interaction with season affected all parameters except *b* fraction of CP and interaction did not affected *PD* of CP. *Chromolaena odorata* had the highest estimated parameters of degradation during the three seasons compared to other browse species. Based on *PD* and *ED*, the plant species followed this decreasing order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. All parameters were positively correlated to CP except the *a* and *b* fractions of DM and CP, respectively, and *lt* of both DM and CP. All parameters were negatively correlated to NDF, ADF and ADL except the *b* fraction and rate of degradation. Condensed tannins (CT) were negatively correlated with all parameters except the *b* fraction and *PD* of DM and CP, and *lt* of DM degradation. Cellulose was negatively correlated with *a* of DM and *lt* of CP degradation.

The broad objective of this study was to assess whether plant characteristics, *in sacco* degradability, *in vitro* gas production, digestibility or chemical composition (NDF, ADF, ADL, CT and CP) could predict the selection of browse species. The parameters were selection index, plant characteristics (spinescence, or leaves phenology), chemical composition, *in vitro* gas production, *in vitro* degradability and *in sacco* degradability

parameters. Most of these parameters were poor for predicting selection by goats of browse species. Spinescence (spn), leaves phenology (phen), NDF, CP and CT accounted for 86% of the variation in selection index (*y)* = -5.91 - 0.01CT - 0.002NDF + 0.02CP + 6.18spn + 2.43phen; (R^2=0.86; n=20; RMSE=0.406; P<0.001; for phen, 1 = evergreen, 0 = deciduous; for spn, 1 = spinescent, 0 = spineless).

It was concluded that *in vitro* gas production, *in vitro* degradability and *in sacco* degradability were poor predictors of selection index of browse species used by goats. Spinescence, leaf phenology, condensed tannins, CP and NDF were predictors of diet selection index and suggested that these plant species have potential to be used as feed supplements. *Chromolaena odorata* has the highest potential as feed protein source in goats. The addition of PEG (tannin binding agent) emphasizes that the inhibitory effect of tannins on rumen microbes was great in tannin-rich feeds.

Thesis output

Published Articles

1. **Basha, N.A.D., Scogings, P.F. and Nsahlai, I.V., 2009.** Diet selection by Nguni goats in the Zululand Thornveld. South Africa Journal of Animal Science, (Supp.1), 39, 33-39.
2. **Basha, N.A.D., Scogings, P.F. and Nsahlai, I.V., 2011.** Effects of season and species on *in sacco* degradability of plant species in the sub-humid subtropical savannah, South Africa. In: Proceedings of the 8th International Symposium on the Nutrition of Herbivores, Aberystwyth, Wales UK, 6-9 September 2011, Cambridge University Press, pp 487.
3. **Basha, N.A.D., Scogings, P.F. and Nsahlai, I.V., 2012.** Diet selection of Nguni goats in relation to season, chemistry and physical properties of browse in sub-humid subtropical savannah. Small Ruminant Research, 102, 163-171.

Conference Abstracts

1. **Basha, N.A.D., Scogings, P.F and Nsahlai, I.V., 2008.** Diet selection by Nguni goats in the Zululand Thornveld. Grassland Society of Southern Africa, 43rd Annual Congress, 21st to 25th July 2008, ForeverSA Resort Badplaas Mpumalanga, pp 65.
2. **Basha, N.A.D., Scogings, P.F and Nsahlai, I.V., 2008.** Diet selection by Nguni goats in the Zululand Thornveld. 10th World conference on Animal Production, 23rd to 28th November 2008, Cape Town, South Africa.
3. **Basha, N.A.D., Scogings, P.F and Nsahlai, I.V., 2011.** The effects of season, species and tannins on gas production kinetics of plant species harvested in different seasons from the sub-humid subtropical savannah, South Africa. Southern Africa Society of Animal Science, 44th Biannual Congress, 11-14 July 2011, Stellenbosch University, Western Cape Province, South Africa, pp 65.

Articles in Preparation

1. Effects of season, species and polyethylene glycol (PEG) on gas production kinetics of forages in the sub-humid subtropical savannah, South Africa.
2. Effects of season, species and polyethylene glycol (PEG) on *in vitro* degradability of forages in the sub-humid subtropical savannah, South Africa.
3. Effects of season and species on *in sacco* degradability of forages in the sub-humid subtropical savannah, South Africa.
4. Relationship between selection index, gas production kinetics, *in vitro* degradability, *in sacco* degradability and chemical composition of forages in the sub-humid subtropical savannah.

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Glossary of abbreviation and symbols

*	Significant ($P<0.05$)
$^{\circ}$C	Degrees centigrade of temperature
µ	Maximum rate of gas production at the point of inflection of the gas curve
A	Gas produce from soluble fraction from *in vitro* fermentation
ADF	Acid detergent fibre
a_{dm}	Ruminal degradable of soluble fraction of the dry matter
Ak	*Acacia karroo*
a_n	Ruminal degradable of soluble fraction of the nitrogen
An	*Acacia nilotica*
Ana	*Acacia natalitia*
ANOVA	Analysis of variance
ApDeg	Apparent degradability
ATP	Adenosine triphosphate
B	Gas produce from insoluble degradable fraction from *in vitro* fermentation
BAS	Browseable trees within species
b_{dm}	Ruminal degradable of insoluble fraction of the dry matter
Bl	Broad leaves
b_n	Ruminal degradable of insoluble fraction of the nitrogen
C	Gas production rate from *in vitro* fermentation
c_1	Degradation rate of gas for soluble fraction (A) from *in vitro* fermentation
c_2	Degradation rate of gas for insoluble fraction (B) from *in vitro* fermentation
$CaCl_2$	Calcium chloride
c_{dm}	Ruminal degradation rate of insoluble fraction of the dry matter
Cell	Cellulose
CHO	Carbohydrates
c_n	Ruminal degradation rate of insoluble fraction of the nitrogen
Co	*Chromolaena odorata*
CO_2	Carbon dioxide
CP	Crude protein
CT	Condensed tannins
C_y	Number of times species „y' was consumed
D	Dry season
Dc	*Dichrostachys cinerea*
Dec	Deciduous plant species
DEF	Degradability efficiency factor arising from *in vitro* fermentation
df	Degree of freedom
DM or dm	Dry matter (oven dried if not stated otherwise)
DMI	Dry matter intake
DOM	Organic matter degradability
D_s	Sum of all observations of all species in the diet
E	Early wet season
ED_{dm}	Effective degradability of the dry matter in the rumen
ED_n	Effective degradability of the nitrogen in the rumen
Ev	Evergreen plant species
F	F ratio
Fl	Fine leaves
g	Gram
GP	Gas production or maximum gas production from *in vitro* fermentation
Gs	*Gymnosporia senegalensis*
h	Hour
ha	Hectare

KCl	Potassium chloride
kg	Kilogram
L	Late wet season
LSD	Least significant difference between means
lt	Lag time for gas production from *in vitro* fermentation
lt_{dm}	Lag time of the dry matter degradability in the rumen
lt_n	Lag time of the nitrogen degradability in the rumen
m	Meter
m^2	Miter square
mg	Milligram
$MgCl_2.6H_2O$	Hydrated magnesium chloride
ml	Milliliter
MPTs	Multipurpose trees and shrubs
MY	Microbial yield from *in vitro* fermentation
N	Nitrogen
Na_2HPO_4	Sodium dihydrogen phosphate
$NaHCO_3$	Sodium hydrogen carbonate
NDF	Neutral detergent fibre
NDF-N	Neutral detergent fibre bound nitrogen
NDS	Neutral detergent solution
ns	Non significant (P>0.05)
OB	Other browse species
Om	Organic matter
P	Statistical significant level
PCA	Principal components analysis
PD_{dm}	Potential degradability of the dry matter in the rumen
PD_n	Potential degradability of the nitrogen in the rumen
PEG	polyethylene glycol
PF	Partitioning factor (Deg/Gas Vol) arising from *in vitro* fermentation
phen	Phenology
r	Correlation coefficient
R^2	Coefficient of determination
RAS	Relative abundance of browseable species
RA_y	Relative abundance of species ‚y'
RMSE	Root of mean square error
SCFA	Short chain fatty acids, also commonly known as volatile fatty acids (VFA)
SE	Standard error
Se	Season
SEM	Standard error of the mean
SI or sindex	Selection index
SI_y	Selection index for species ‚y'
Sm	*Scutia myrtina*
Sp	Plant species
Spce	Spinescent plant species
Spl	Spineless plant species
spn	Spinescence
SSA	Sub-Saharan Africa
t	T-test
$T_{1/2}$	Time taken to produce half maximum gas volume from *in vitro* fermentation
TrDeg	True degradability

Chapter 1

Introduction and literature review

1.1 Background

Diet selection is an important requirement for evaluating the effects of herbivores on their environments; this would increase understanding of plant–herbivore interactions and help to achieve optimal management strategies and profitable animal performance. The varied differences in diet selection among browse species might be ascribed to differences in palatability. This has been commonly known to be affected by plant chemistry (Tixier *et al*., 1997), plant morphology (spinescence) (Schindler *et al*., 2003; Hanley *et al*., 2007) and plant phenology (Shipley *et al*., 1998; Watson & Owen-Smith, 2002), digestibility and intake. Moreover, the patterns of diet selection are not regular in space or time, which may be due to seasonal changes in forage availability (Edenius *et al*., 2002) or differences in chemical and physical defence (Illius *et al*., 1999; Dziba *et al*., 2003a). Several studies have assessed the relationship between diet selection, and plant chemistry, morphology and phenology traits, but there is little information of these relationships in savannahs.

It has been recognized that plant palatability affects dry matter intake, that may be due to the high level of secondary plant metabolites, such as condensed tannins (CT) (Alonso-Díaz *et al.,* 2008). Additionally, high fibre may reduce foraging efficiency and extend the ingesta retention time in the rumen. That may be due to their low breakdown rate by rumen microbes. Furthermore, ruminants require sufficient dietary fibre for standard rumen functions which is associated with secretion of enough saliva and optimal pH (Church, 1988). Fibre has an important function in ruminants because it limits intake and maintains normal fermentation in the rumen (Ndlovu & Nherera, 1997; Lu *et al*., 2005). However, high phenolic concentrations may have negative effects on the nutritive value of forages by changing their palatability, reducing intake and digestibility (Kumar & D'Mello, 1995). High levels of CT in plant reduced palatability that may be due to astringency and short-term post-ingestive malaise (Provenza, 1995; Silanikove *et al*., 2001). Some forages are known for their high content of CT (Monforte-Briceño *et al*., 2005). Generally, CTs are associated with negative effects in ruminants (Kumar & Vaithiyanathan, 1990). These

negative effects are related to their affinity to form complexes with dietary proteins, carbohydrates and minerals (McSweeney *et al*., 2001). High levels of CT in the diet decrease digestibility and voluntary DM intake (Decandia *et al*., 1999; Silanikove *et al*., 2001). This could be attributed to CT interactions with proteins, digestive tract enzymes, microbes and digestive tract mucosa (Silanikove *et al*., 2006). On the contrary, tannins in low to moderate concentrations have beneficial effects (Hoste *et al*., 2006), which are related to suppression of bloat in ruminants (Jones *et al*., 1973), protection of dietary proteins from microbial degradation in the rumen by formation of protein tannin complexes (Barry *et al*., 1986; Min & Hart, 2003), their anthelminthic properties and anti-oxidant effects (Saura-Calixto *et al*., 2007). These tannin-protein complexes might mostly disassociate in the abomasum at a low pH and release bound protein, which become available for digestion. However, the effects of tannins on digestibility (*in vitro* and *in sacco)* of indigenous tree/shrub forages in African savannahs remains unknown.

In vitro gas production techniques have become accepted for evaluating forage digestion features (Groot *et al*., 1996; Getachew *et al*., 1998; Theodorou *et al*., 1998; Campos *et al*., 2004; Dijkstra *et al*., 2005). Moreover, the *in vitro* gas production analysis is the best technique among biological ones currently used to assess the negative effects of tannin on nutritive value of feed. Tannin binding agents, such as polyethylene-glycol (PEG), have been applied to reduce the anti-nutritional effects in browse species by forming tannin-PEG complexes (Makkar *et al*., 1995a; Silanikove *et al*., 2001). Furthermore, *in sacco* degradability is a major evaluation technique to determine the nutritive value of forages (Ørskov & McDonald, 1979), ranking browse trees in terms of quality (Mehrez & Ørskov, 1977) and for evaluating the digestive abilities of ruminant species (Migongo-Bake, 1992). The *in sacco* technique may give direct information on the effects of browsing on animal performance.

Depending on the narrative above, the question that comes to mind is: Is it possible to predict the diet selection of goats using the nutritive value of the feed? This thesis will attempt to answer this question.

1.2 Study objectives

The broad objective of this study was to determine the patterns of diet selection of goats on pasture woody species in sub-humid subtropical savannah, South Africa and predict the diet selection using chemical composition, *in vitro* gas production, *in vitro* degradability and *in sacco* degradability.

The specific objectives of this study were: to:

1) Explore diet selection of goats foraging in different seasons in a sub-humid subtropical savannah.
2) Investigate the relationships between diet selection and various physical, chemical and phenological features.
3) Determine the *in vitro* and *in sacco* nutritive characteristics of browse species.
4) Determine the effect of tannins on *in vitro* nutritive characteristics of browse species.
5) Derermine the relationship between diet selection and chemical composition, *in vitro* gas production, *in vitro* degradability and *in sacco* degradability.

1.3 Hypotheses

To achieve the above objectives, we tested the following hypotheses. Seasonal variations in availability and quality of forage in sub-humid subtropical savannah may change diet selection (Abate, 1996) and could affect the degradability of browse species (Winrock, 1992; Larbi *et al*., 1998; Ramírez-Orduña *et al*., 2003; Camacho *et al*., 2010). Physical traits may affect diet selection as long as spinesence (Cooper & Owen-Smith, 1986; Gowda, 1996; Papachristou *et al*., 2003) and leaf morphology (Shipley *et al.*, 1998; Dziba *et al.*, 2003a, Wilson & Kerley, 2003a) have been known to affect the foraging behaviour of mammalian herbivores. Inherent differences in leaf chemistry among different plant species might affect diet selection (Scogings *et al.*, 2004) and could influence the rumen degradability (Nsahlai *et al.*, 1994; Ndlovu & Nherera, 1997; Meissner *et al*., 1999; Van Hoven, 2000). Plant chemical composition and nutritive value (*in vitro* and *in sacco* degradability) could predict diet selection since the chemical composition and degradability links to the feed palatability and rumen retention time (Provenza & Villalba 2006).

1.4 The use of trees and shrubs in ruminant nutrition

The strategy of including forages into ruminant feeding systems is not yet completely developed. Forages have potential to cover the harsh nutritional shortages that ruminants face within tropical production systems (Devendra, 1990). Low growth rates and low animal production may be partly due to lack of adequate feed throughout the year, which is possibly the major factor particularly during the dry season in subtropical and tropical regions (Makkar & Becker, 1998; Sawe *et al.*, 1998). Certainly, improved animal performance has been reported in response to the use of high quality forage legumes as supplements for ruminants fed on low-quality roughage diets (Abarca, 1989; Gonzalez *et al.*, 1996; Aganga & Tshwenyane, 2003), possibly due to high protein level and intake of these forages compare to low CP contents and intake of tropical grasses. This is linked with short retention time of legume material in the rumen, and high mass for legume forage in the rumen (Humphreys, 1991). There are many advantages to offer legumes as supplements to poor quality diets in smallholder production systems, such as availability on the farm, accessibility, provision of variety in the diet, reduction in the requirements of purchased concentrates, reduced cost of feeding (Devendra, 1990) and improved soil fertility due to the nitrogen fixation ability of legumes (Brewbaker, 1986).

1.5 Diet selection

Herbivores earn energy from feeds for their maintenance and production (Provenza, 1995; Wilmshurst *et al.*, 2000), beside other nutrients such as proteins, amino acids, vitamins, and macro and micro elements (Schmidt-Nielsen, 1997). Previous studies pointed out that most herbivores forage to maximise energy intake. Herbivores preserve their fitness by optimal feeding to select the greatest amount of energy and/or other nutrients (Wilmshurst *et al.*, 2000; Mutanga *et al.*, 2004; Whelan & Brown, 2005; Nastis, 2007). Jansen *et al.* (2007) explained that diet selection by mammalian herbivores is balanced by maximizing nutrient intake and minimizing of plant secondary compounds, or by satiety.

Herbivores are able to select from a range of feeds to provide nutrients required to sustain their essential body functions (Wilmshurst *et al.*, 1999a, b; Forbes, 2007; Nastis, 2007). Even with a greatly concentrated feed that fulfils the individual's nutrient requirements in few bites, it will continue to feed beyond this point because its digestive tract is still largely

unfilled. Herbivores have to base their diet selection to choose feeds which satisfy their nutritional needs and make them sense when they are sated (Provenza, 1995). The methods for attaining these goals are uncertain (Stephens & Krebs, 1986). The knowledge of a function of selecting food by small ruminants might be useful to present best management of pastures. This knowledge based on feeding behaviour is a key to understand plant-herbivore interactions (Ungar, 1996; Dziba *et al*., 2003a), proficient management of pasture systems and ultimately gainful livestock enterprises (Ungar, 1996).

1.6 Factors affecting goat feeding behaviour and diet selection

Voluntary intake of feed by herbivores is the amount consumed during a specified period of time when there is free access to the feed, however, which feeds are selected depend on many factors associating with the animals, the environment, and the feed (Schülke *et al*., 2006). Factors such as the physiological stage of animal, the foraging experience in the past, the surrounding conditions, the palatability of browse species, and the accessibility of browse material (Owen-Smith, 1999; Nastis, 2007), the type of animal species and their adaptation depend on the anatomy and physiology (Robbins *et al*., 1995). For ruminants, learning feeding habits through taste and smell of food are keys in the feeding behaviour (Forbes, 2007; Nastis, 2007). Plant species have defence lines against foraging by herbivores, whilst herbivores have the ability to manipulate these defences and forage on these plant species (Van Soest, 1994).

1.6.1 Factors related to animals

According to the animal anatomy and its grazing/foraging abilities, herbivores are mainly classed into grazers and browsers; grazers feeding predominantly on grasses and browsers mainly feeding on browses which consists of trees and shrubs. Furthermore, many studies have classified mixed feeders which feed on mix proportions of grass and browse (Hofmann, 1989; Van Hoven, 2000).

An animal's body size has a primary influence through its metabolism and hence its food and energy needs (Stokke, 1999). Animals require energy to perform their different functions, and the utilisation of energy always depends on the animal energy metabolism (Schmidt-Nielsen, 1997). The body energy demands are determined by metabolic rate,

which is associated to the body mass (Illius & Gordon, 1992). It was found that a negative relationship exists between energy utilisation per unit of body mass and the animal's mass. The metabolic rate is also influenced by the reproductive stage, lactation, mobility, weather conditions (Owen-Smith, 1999), and growth (Dietz & Drent, 1997). There is a negative relationship between body mass and metabolic rate, and accordingly the daily food intake (portion of body mass) decreases with increasing body size (McNaughton & Georgiadis, 1986). Thus small animals need relatively more energy in order to meet their high mass-specific energy demands. In accordance with Owen-Smith (1999) and Nastis (2007), it is expected that animals select a feed that is good in nutritive value and can offer a high energy. The fraction of good quality feeds in the diet increases with a decrease in the size of the animal (McNaughton & Georgiadis, 1986).

The rate of food intake is determined by the number and mass of bites per minute, which herbivores can crop from pasture (Welch & Hooper, 1988; Barboza *et al*., 2009). A bite or nibble is breaking off or picking up a piece of forage (Agreil & Meuret, 2004). However, bite size differs widely depending on what was being eaten, and is complicated to measure precisely under field conditions (Oliveira & Silva, 2007). Herbivores with small mouths are more skilled at being selective of plant parts compared with those species with large mouths (Jarman, 1974). Grazing and browsing ruminants vary in muzzle size and shape relative to their body size (Janis & Ehrhardt, 1988). Grazers have moderately broad muzzles which favour short grass over browse species. Wildebeest have broad muzzles, and thus prefer short creeping grass and low-growing forms (Owen-Smith, 1999). Browsers are able to eat leaves from thorny plant species because of their narrow muzzle. Variations in the muzzle thickness influence the performance of animals grazing on swards of different structure. For instance, the wildebeest can continue to crop at sufficient rate of intake, even when the grass is short (50 mm) (Owen-Smith, 1999). However, Dziba *et al*. (2003b) postulated that bite size and intake rate is probably bigger due to the wider mouth size for Boer goats compared to Nguni goats. According to the lips of different animal species, the anatomical adaptations that allow some animals to graze and others to browse selectively is revealed. Selective feeders have organs to enable them to crop leaves between thorns by moving their upper lips and tongues to bring the bite into the mouth (Welch & Hooper, 1988; Illius *et al*., 1999).

Ruminants have four unique chambers three of which are for fermentation (rumino-reticulum) situated in front to the true stomach (abomasum) (Sjaastad *et al.*, 2003). The chambers are the rumen (large part), reticulum, omasum and abomasum (Hofmann, 1988; Sjaastad *et al.*, 2003). The rumen includes the new meal separate from the meal that has been taken previously. In ruminants, amounts of consumed food depend on the capacity of their rumen, and largely determine the differences in intake of different species and their forage. The ruminant animal's digestive processes and the outflow of ingesta through the digestive tract influence the level of rumen fill. The capacity of digestive tract of ruminants is greatly bigger than in monogastric animals; it provides space for the ruminant to forage great amounts of feed until a certain level of rumen fill (Hofmann, 1988). The degree of forage breakdown in the rumen is strongly related to the abundance and character of the fibre fractions (cell wall) in the forage, since these fractions depress fermentation and outflow rate of particles. When Gordon and Illius (1996) offered the same food to different animals with various body sizes, they concluded that a lower fermentation rate of digesta in the rumen is associated with large animals.

Concentrate selector animals have a lesser ability to digest fibre because their digesta in gastro-intestinal tract pass fairly rapidly. Lechner-Doll *et al.* (1995) found that concentrate selectors usually select forage with low fibre. These animals are able to select from the plant only the parts that are highly digestible and necessary to their survival (Duncan *et al.*, 2003). The feeding policy of the roughage or bulk feeders can be considered as "more is better" and "quantity, not quality". Grazers have large rumens that allow them to keep the slow-fermenting grass fibres for enough time to extract most of the digestible energy (Van Soest, 1994). These animals get significant energy from digesting the fibre fractions of plants (Lechner-Doll *et al.*, 1995). Mixed feeders are less selective in what they consume than are the selectors, but they are unable to constantly eat large amounts of highly fibrous feeds. Both the quantity that they can consume and the length of time that feed is kept in the rumen are restricted by the capacities of their digestive systems (Owen-Smith, 1999). High quality food is relatively rapidly digested and passes down through the animal's digestive tract and as such stimulates more food intake and animal production (Lechner-Doll *et al.*, 1995). Animals are able to buffer food intake by anatomical or physiological ways. Anatomical buffering are secretion from organs such as mouth pouches, the crop and rumen. Physiologically, they buffer food by releasing energy from fat deposits in the body and the exchange capability of metabolic energies; for example, energy may be obtained

by catabolizing the proteins when inadequate energy is consumed (McDonald *et al*., 2002). It has been defined that voluntary intake is a function of both the food and which digestive system type the animal has (Schmidt-Nielsen, 1997; Owen-Smith, 1999).

1.6.2 Factors related to plants

Feeding behaviour can be categorised into habitat preference, feeding preference and behaviour patterns (Nastis, 2007). Feeding preference functions is passing through the selection of foliage covers, taxonomic classes of plants, plant species and plant parts. The main factors that affect the feeding behaviour of animals are availability, acceptability, digestibility and chemical composition of food (Goetsch *et al*., 2010). Feeding behaviour reflects the relation between the animal and the vegetation in its environment (Goetsch *et al*., 2010). This behaviour can be changeable for those animals that have ability to apply different feeding strategies to keep themselves under changing and unfavourable environmental conditions (Nastis, 2007). Some capitalize on the quality of the feed by cautious selection among the feeds; some eat large amounts of fibrous feeds, while others approve an intermediate approach to diet selection (Welch & Hooper, 1988). The inherent anatomical, physiological and behavioural aspects of the ruminant digestive tract varies among animal species and have a basic influence on their feeding patterns. Generally, plant selection by animal from plant community (veld) is mostly linked to those elements of a site that affect its ability to harvest nutrients. Nastis (2007) recognised that forage quantity and quality was strongly associated with the ratio of period of time spent foraging in the pasture relative to the area used within the whole landscape. An animal's feeding station is recognized when it stops walking, moves the direction of its head and bites a plant. Specific sensory signals that let the animal to stop searching and to select a particular species or combination of species it regard as profitable. The pattern of feeding stations is closely related to the distribution and abundance of plant species in the pasture, the size of the pasture contents. Hirata *et al*. (2010) mentioned that foraging behaviour time can be distributed into search, feeding stations, biting whilst at a feeding station. Illius *et al.* (1999) suggested that the nutritive value based on chemical and nutrient composition of plant that are fed on, their digestibility, and the rate of feed intake are the main factors that determine animal performance.

Studies have been done on the effect s of plant communities on ingestion rates (e.g. Arnold, 1981; Forbes *et al*., 1985). Gordon and Illius (1996) concluded that feed characters rather than anatomical adaptations are of greater importance for the nutritional ecology of some ruminants. Partitioning of feeding stations by searching seems to be a regulation behaviour linked with forage quality. The main factors influencing diet selection of herbivores is quantity and quality of feed available. This is confirmed by the reality that the diet composition of any herbivore varies over time (season) and space (location) in response to variations in quantity and quality of available food (Dziba *et al.*, 2003a). The quantity and quality of forage consumed does not just determine the production of an animal species and reflect its behaviour and habitat (Milne, 1991), but also provide reliable information on the value of those plants in addressing the nutritional needs of the individual.

1.6.3 Plant species composition

Veld is naturally heterogeneous and contains different species with varying acceptability and palatability, which is determined by the forage composition (Aregheore *et al*., 2006; Skarpe & Hester, 2008). Preference, which is effectively behaviour, involves relative selection of particular plant species from among different species. The preference category of a plant species is mostly based on its abundance, its morphology and phenological traits, the range of species on offer and the herbivore species in question (Osolo *et al*., 1996). In browse herbivores, diet selection relies on preferred and principal feeds. Preferred plant species are consumed in a higher ratio than their abundance in the feeding area, regardless of the degree to which the exact plant species adds to the entire diet. Rather, principal species are those with a great contribution to the total diet selected by an animal, regardless of its preference, relative to other plant species on offer (Dziba *et al*., 2003a). The preferred species improve the diet nutritionally, resulting in enhanced animal performance while the principal species are not usually as high in nutrients as the preferred species but offer the animal chance to maximize instantaneous intake rates (Osolo *et al*., 1996). Diet selection can also apply to determine dietary preferences for different forage species. Dietary preference is the ratio between the abundance of a plant species in the diet of herbivore, compared to its abundance in the veld (Tanentzap *et al*., 2009).

1.6.4 Plant acceptability

Physical plant defences such as spines, thorns and prickles have special forms to work as defences against herbivorous animals and have been reported to influence the foraging behaviour of animals (Papachristou *et al*., 2003). Spinescence has different types of forms and followed by varying effectiveness against different animal species, thus, it may establish spine-animal inteactions. Gowda (1996) reported that less foraging rates were recorded when goats browsing on species defended with thorns, spines or prickles compare to those that were spineless. Increasing in spine length may increase protection of leaves of browse species from ungulates such as goats and giraffe (Young *et al.,* 2003). Moreover, spine density has a negative relation with foraging rate because of protecting the twigs by spine density (Gowda, 1996). The rank of leaves and stems on the plant may control the size and rate of bites (Shipley & Yanish, 2001). The structure of some forms of spinescence may added more function; for example hooked thorns may hold on some parts of animal (lips, tongue, ears and skin) and thus need animals to warily manipulate the plants in the mouth to avoid pain and injuries. On the other hand, acceptability of woody plant may be limited by growth stages of plant, soil nutrient, light availability and previous defoliation history which could influence the plant (Stamp, 2003).

The circumstances under which a plant grows also influence its acceptability. Neighbouring plant species may change a plant's acceptability by masking its chemical cues and restricting the animals from foraging nearby (Palmer *et al*., 2003) due to their smell, or by physically reducing access to the other plant. The relative abundance and preference for other plant species growing in the same area may also influence acceptability. In pastures where there are manure pads, there is a zone of aversion around each manure deposit where animals will not graze unless no other feed is available (Welch & Hooper, 1988). This unacceptability might be due to the smell or taste of the grass that is closely associated with the dung pad.

1.6.5 Plant chemical composition

Plants contain various chemical components: (i) carbohydrate structure (cellulose, hemicellulose) which are indigestible or less digestible, (ii) lignin which is indigestible and (iii) cell contents (proteins and soluble carbohydrates), which are readily digestible (Van Soest, 1967; Hall *et al*., 1982). These chemical substances are nutrients for herbivores. Furthermore, plants have primary and secondary substances; the primary substances include starches, lipids, proteins, vitamins, and minerals (Hall *et al*., 1982) which play a significant role in the metabolism of the animal while secondary substances include glucosides, saponins, tannins, alkaloids, essential oils and organic acids, which may have no important role in the metabolism of the animal. The plant species vary in chemical composition across seasons, resulting in herbivores showing preference for a particular plant in relation to its chemical constituents (Basha *et al*., 2012). Secondary components offer plant resistance to feeding animals by providing particular chemicals (Bennett & Wallsgrove, 1994). They are assumed to have little direct negative effect on herbivore fitness and a deterrent effect on herbivore foraging (Roy & Kirchener, 2000). Animals have enhanced their ability to tolerate secondary plant components or even detoxify those (Robbins *et al*., 1987a). Proteins, vitamins and minerals are vital substrates in animal's feed and are requisite in a correct balance for animals to perform satisfactorily. Insufficient amounts and imbalances of minerals in forages may lead to physiological disorders and restrain an animal's performance (Bicknell, 1995). Proteins are the limiting factors to animal performance in the veld (Meissner *et al*., 1999; Abdou *et al.,* 2011). Moreover, the protein requirements by animals are affected by the species (Meissner *et al*., 1999), age (Meissner *et al*., 1999; McDonald *et al*., 2002), physiological functions (growth or lactation) (Owens & Zinn, 1988; Meissner *et al*., 1999) and seasons (Fourie *et al*., 1986). In general, ruminants require low amounts of CP (70-80 g kg^{-1} DM), and high performance animals require relatively high level of CP (130-140 g kg^{-1} DM) (Owens & Zinn, 1988; Meissner *et al*., 1999). Crude protein is assumed to be the main factor affecting diet selection of woody plants (Ganqa *et al*., 2005). However, tannins are considered as a main anti-nutritional factor, which is discussed below.

1.6.6 Tannins

Tannins are polyphenolic compounds present in a wide range of plant species such as *Acacia* spp (Bryant *et al*., 1992; Jackson *et al*., 1996) as well as pasture species such as *Lotus* spp (Terrill *et al*., 1992a). Tannins are divided according to their chemical structure into two groups: hydrolysable (HT) and condensed tannins (CT) (Kumar, 1983; Bennick, 2002). The two types of tannins have various nutritional effects in ruminants.

Hydrolysable tannins are molecules containing a carbohydrate, generally D-glucose, as a central core (Clifford, 2001). The hydrolysable carbohydrates have formed as polymer of phenolics such as gallic acid and ellagic acid. Generally, HT are found in small percentages in plants. Hydrolysable tannins are hydrolyzed by tanninase enzymes into taragallotannins and caffetannins. Hydrolysable tannins can form toxic compounds such as pyrogallol, which cause hyper emission of gastric mucus and histo-pathological lesions in the gastrointestinal tract of ruminants (Mitjavila *et al.*, 1977). High quantities of HT have been responsible for poisoning in animals. This is caused by damage of the detoxifying enzymes in the liver by HT, which causes liver necrosis, kidney damage and high mortality in ruminants (Reed, 1995; Krebs *et al*., 2003). In addition, HT affect monogastric species by reducing growth rate, protein utilization and causing damage in the digestive tract mucosa (Ahmed, 2010).

Condensed tannins (CT) are widely distributed in several kinds of plant species and legume pasture (Mueller-Harvey & McAllan, 1992). Condensed tannins are mostly found in many plant parts such as stem, fruit, leaves and roots. Condensed tannins consist of many flavan-3-ols bound by carbon-carbon bonds, which further form flavanoid units. The complexity of CT depends on the variation in the flavanoid units among constituents and within sites for interflavan bond formation. Anthocynidins are produced upon heating of proanthocyanidins (PAs) in acidic alcohol solutions. Notably, PAs are derived from the acid-catalyzed oxidation reaction (Bennick, 2002). Anthocynidins are responsible for the colours observed in flowers, leaves and fruits. The problem of using browse species as alternative protein sources by ruminants is the occurrence of CT. Therefore, condensed tannins and their tannin-protein interactions, they become important because of their influences on animal performance.

1.6.6.1 Mechanisms of tannin-protein complexes

Condensed tannins form tannin-nutrient complexes with some plant compounds such as proteins, polysaccharides, nucleic acids, steroids, alkaloids, saponins (Mueller-Harvey & McAllan, 1992). It has been suggested that the essential mechanism of these interactions is the same. Tannins may interact with other molecules through hydrogen bonding, hydrophobic bonding or ionic bonding. It has been accepted that the hydrophobic and hydrogen bonds are the main bonds in the complexation of tannins with other molecules. Tannin-protein complexes are structured by hydrophobic and hydrogen bonds which are reversible (Hagerman *et al*., 1998). Hydrogen bonding is the primary mode of attachment employed by tannins to bind proteins (Liu *et al*., 2006). The relative importance of hydrogen bonding versus hydrophobic bonding depends on the nature of both the protein and the tannin (Siebert *et al*., 1996).

1.6.6.2 Factors affecting the formation of tannin-protein complexes

Characteristics of both tannins and proteins can affect the tannin-protein complex formation (Butler, 1989; Mueller-Harvey, 2006). Molecular weight of tannins can influence their ability to precipitate proteins. Murdiati *et al*. (1992) suggested that phenolic compounds of low molecular weight form unstable bonds with proteins, while those of high molecular weight are ineffective as tannin agents. The molecular weight of CT varies widely according to factors such as forage species and plant tissue (Reed, 1995). Additional work has shown that large molecular weight of CT is likely to lose their protein-precipitating capacity (Kumar & Vaithiyanathan, 1990), which might have inference for N use by ruminant animals. Indeed, it is apparent that the molecular weight of CT provides a very significant role in determining their effectiveness as protein-precipitants.

Tannin-protein binding can be fairly specific for both the protein (Hagerman & Butler, 1981) and the tannin (Asquith & Butler, 1986); there are protein characteristics that dictate their affinity for tannins. A protein with a high affinity for tannin may be selectively bound and precipitated out of a large excess of proteins with lesser affinity. Hagerman and Klucher (1986) suggested that proteins differ widely in their affinities for specific tannin. Those proteins that bind strongly to tannins have properties that include high molecular

weight, open and flexible structures (Mueller-Harvey & McAllan, 1992). Furthermore, a common character of protein with a high affinity for tannins is their richness in the amino acid proline (Hagerman & Butler, 1980b). Salivary proteins have a high affinity for tannins in herbivores (Austin *et al*., 1989; Mole *et al*., 1990; Vaithiyanathan *et al*., 2001) that may be due to a high proline in salivary proteins (Shimada, 2006) which may increase the ability to form strong hydrogen bonds by increasing accessibility of the peptide linkages (McArthur *et al*., 1995). Moreover, salivary proteins have been recognized by two groups namely proline-rich proteins and histatins, are effective precipitators of tannins (Da Costa *et al*., 2008). Tannin binding salivary proteins are likely to secrete in response to diet selection by herbivores (Hagerman & Robbins, 1993) and have been noticed when animals consume tannin-rich feeds (Shimada, 2006).

There are some factors that affect the formation of tannin-protein complexes. One of these factors is polyethylene glycol agent (PEG). Ben Salem *et al*. (1999) and Guimarães-Beelen *et al*. (2006) treated leaves from different tropical legumes with PEG4000 to bind soluble CT and then determined the astringency of the unbound tannins. As the quantity of PEG increased, the concentration of 70% aqueous methanol-extractable tannins decreased. Similar results have been found in terms of fibre bound tannins, so that the total quantity of tannins in the sample remained relatively constant (Guimarães-Beelen *et al*., 2006).

1.6.6.3 Dissociation of the tannin-protein complexes

Condensed tannins have usually been considered to reduce plant preference by digestion inhibition (Barahona *et al*., 1996; Guimarães-Beelen *et al*., 2006) because they can form and make inactive digestive enzymes (Bae *et al*., 1993; Jones *et al*., 1994) and precipitate dietary proteins (Guimarães-Beelen *et al*., 2006). Precipitated protein would most likely be less digestible than soluble protein, thus the animals would get insufficient dietary protein from tannin-rich plants. Under this assumption, disruption of the complexes between tannins and other molecules will counteract the potentially deleterious effects of tannins. It is assumed that detergents might disconjugate tannin-protein complexes. The detergents include non-ionic, ionic, cationic detergents (Oh *et al*., 1980), dioxane, dimethylformamide (Hagerman & Butler, 1980a) and acetone (Hagerman, 1987). Different tannin-binding agents have been used for different functions: estimation of tannins (Makkar *et al*., 1993), extraction of enzymes (Badran & Jones, 1965) and inhibiting the effects of tannin-rich

foods and feeds (Barahona *et al*., 1996; Guimarães-Beelen *et al*., 2006). These agents involve polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP), with various range of effectiveness (Makkar *et al*., 1995a); all these agents have been shown to bind plant tannins. Makkar *et al*. (1995a) assumed that tannins bind favourably to PEG, which replace protein in tannin-protein complexes. In addition, Jones and Mangan (1977) and Jones *et al*. (1994) reported that with PEG, tannin-protein complexes are insoluble at pH range from 4 to 6.5, however, more than 95% of protein was discharged at pH 1-3 and 30 % of the protein was discharged by PEG at pH 8-8.5. In term of ruminant nutrition, as long as the rumen pH falls between 5.5 to 7.2, great amount of tannin-protein complexes would then escape the rumen and protein would be released from tannin-protein complexes in the post-rumen and digested readily by the gastric (pH 2.5) and pancreatic (pH 8-9) secretions (Owens & Goetsch, 1988). If this is right, tannins could be a realistic material for protecting protein from rumen degradation and increasing the amount of available dietary protein that reaches the duodenum. This relationship might not be true in the small intestine, since the pH of the digesta in the abomasum was about 3-4 and increased to about 5.5 at the beginning of the small intestine and reached maximum pH 8.0 (Owens & Goetsch, 1988). In addition, digesta would be in acidic environments for short time (20-35 minutes) in the abomasum.

1.6.6.4 Effects of tannins on rumen micro-organisms

Within the tannin types, the condensed type is one which is structurally most complex (Terrill *et al*., 1990), which possibly appears to be extremely difficult to digest (biodegradation) or need more specialised conditions, enzymes and/or micro-organisms. However, there is confirmation of the hydrolysable tannins bio-degradation via some rumen micro-organisms (Nelson *et al*., 1997).

The potential responsibility of deterring herbivory may cause tannins to deter micro-organisms to attack the plant tissue, either by increasing resistance against pathogens or by protecting essential tissues such as wood against crumble (Guimarães-Beelen *et al*., 2006). Tannins have been recognised to be poisonous to fungi, bacteria and yeast. In the ruminant, micro-organisms degrade fibre, but tannin toxicity may hold great nutritional significance. Thus, the correct evaluation of the effects of tannins of legume and shrub tree on rumen micro-organisms is significant. An evaluation of toxicity could be achieved by identifying

bio-chemical characteristics of the metabolism of particular microbes, for instance fibre degradation, and production of nitrate and methane (Scalbert, 1991). In several studies, a negative association between plant tannin content and degradability of protein (Rittner & Reed, 1992; Miller & Ehlke, 1994; Kaitho *et al*., 1998c; Salawu *et al*., 1997b), fibre, (Terrill *et al*., 1989; Reed *et al*., 1990; Wiegand *et al*., 1995; Barahona *et al*., 1997; Ben Salem *et al*., 1997b) and dry matter (Terrill *et al*., 1989; Wang *et al*., 1994; Stienezen *et al*., 1996; Kaitho *et al*., 1998b) by ruminal micro-organisms has been reported. Consequently, negative *in vitro* digestibility of NDF in tropical shrub legumes high in tannins has been observed (Rittner & Reed, 1992). Soluble tannins bind to elements of the cell wall by forming insoluble complexes. These complexes might be indigestible or inaccessible to bacterial enzymes.

The tannins antimicrobial activities (condensed and the hydrolysable) were investigated by Henis *et al*. (1964), who studied the effects of carob pods water extracts and tannin acid on the growth of numerous micro-organisms. Both extracts and tannin acid inhibited the micro-organisms. In the same study, it was seen that gallic acid and catechol in tannins structure were less efficient in deterring the growth of microbes than the polymeric compounds. The effects of tannins on microbial growth could be due to its type and concentration as well as the susceptibility of the micro-organisms involved. Some researches have reported that tannins can also negatively influence the rumen micro-organisms and/or deter or modify their enzymatic functions. For instance, *in sacco* studies showed that the activities of micro-organisms were significantly lower in the tannin-rich legumes as compared to the one treated with PEG (Guimarães-Beelen *et al*., 2006). Jones *et al*. (1994) investigated the interactions of tannins (three sources) and four strains of ruminal bacteria, and observed variation among the bacteria in the amount of tannin required to inhibit their growth and among the tannins in their capacity to inhibit microbial growth. *In vivo*, the effects of tannins on rumen microflora growth can be determined by evaluating protein degradation and other nutrients in the rumen, and generally, the presence of tannins has been related to decreased availability of nutrients to rumen micro-organisms.

A different helpful estimator of microflora growth is the quantity of microbial N that flows to the duodenum of ruminants fed tanniniferous diets. Nevertheless, this measurement is restricted by our ability to discriminate between microbial, endogenous and feed N in

duodenal digesta (Reed, 1995). It has been suggested that tannins might improve microbial yields, possibly because of increase endogenous nitrogen recycling. Moreover, a change in micro-organism populations in the presence of tannins can provide an indication of the influence of tannins on rumen microflora. Several researches have assumed a positive relationship between tannins and ruminal protozoan populations. Patra and Saxena (2009) found higher protozoan numbers with high tannin diets compared to low tannin diets. However, when high tannin diets were treated with PEG, the result showed lower protozoan numbers in sheep (Ben Salem *et al*., 1997b; Animut *et al*., 2008). Increases in protozoan counts might result in decreasing bacterial numbers, but there is no measurement of bacterial growth taken in these two studies. However, Wang *et al*. (1994) reported higher protozoan counts in animals fed on *Lotus corniculatus* and PEG as opposed to those fed on the control diet.

To evaluate the effect of tannins on rumen microbes, there are huge variations between both the *in vitro* and *in vivo* evaluating studies that may refer to many factors. The major factors are dilution and how tannins apply their antimicrobial effects on rumen microbes. Patra and Saxena (2011) reviewed that tannins might achieve their antimicrobial effects through different ways: by deterring the extracellular microbial enzymes, reducing substrate accessibility to microflora, action on microbial membranes by inhibition of oxidative phosphorylation or influencing the integrity of membranes. Of these ways, the inhibition of oxidative phosphorylation is not expected to happen in anaerobic rumen microflora. Butler (1989) assumed that under *in vivo* circumstances digestive enzymes might maintain their function in the presence of tannins, likely due to detergents and unfavourable pH conditions, all of which would prevent tannins from binding protein. In the ruminant, a considerable decrease in extractability of tannins occurs during the chewing, representing small amounts of soluble tannins, which is able to affect microbial enzymes in the rumen (Mehansho *et al*., 1983). In addition, it has been confirmed that some animal species have the capability to produce proline-rich salivary proteins with high affinity for binding tannins, which may lead to further reduction of extractable CT concentrations in the rumen. Thus, it may show that the effect of tannins on microbial activity is mainly by substrate-tannin complex formation (Smith *et al*., 2005). Furthermore, many researchers have reported that, in the occurrence of tannins, numerous bacteria undergo morphological changes (Kumar & Vaithiyanathan; 1990; Jones *et al*. 1994). As a last consideration, it is obvious that the rumen microflora, as an entire and individual

microbial species of the rumen, possess huge flexibility that helps them to live in the face of high concentrations of tannins. It appears that the tannin could cause changes in composition of the rumen microflora.

1.6.6.5 Effects of tannins on nitrogen metabolism

Ruminants utilise protein more efficiently if it is prevented from bacterial deamination in the rumen (Ulyatt *et al*., 1975). Precipitation of protein by the structure of insoluble tannin-protein complexes has been shown to protect dietary protein from ruminal fermentation (McNabb *et al*., 1996; Wang *et al*., 1996b; Min *et al*., 2000; 2005; Al-Dobaib, 2009). These complexes tend to form during mastication; this suggestion was confirmed by the results of Terrill *et al*. (1992b), who found a significant decreasing of solvent-extractable CT in plant tissue after chewing. This decrease offset an increase in the formation of protein-tannin and fibre-tannin complexes (Terrill *et al*., 1992b). Prevention of protein in tannin-protein complexes from deamination by microbial enzymes is really a potentially helpful feature of tannins. However, protein that is not degrading in the rumen but escapes to the small intestine may not become available there (Patra & Saxena, 2011). Certainly, it has been shown that the digestibility of protein in plants containing large quantities of tannins is low and that the decrease in protein availability is related to the protein-precipitating capacity of tannins (McNabb *et al*., 1996; Min *et al*., 2005).

Any explanation of the impacts of tannin on ruminal degradation of nutrients has to basically limit the influences of tannin on ruminal micro-organisms and their enzymatic activity. Tannins suppress microbial enzymatic activity (Scalbert, 1991; Nsahlai *et al*., 2011). Several studies have reported that tannins are effective in preventing proteins from enzymatic and ruminal denitrogenation (Miller & Ehlke, 1994; Ngwa *et al*., 2003; Nsahlai *et al*., 2011). In addition, *in vitro* study showed a negative relationship between protein degradability and concentration of CT in plant, which was clarified by the formation of insoluble tannin-protein complexes (Silanikove *et al*., 2001). However, addition of tannin-containing cottonseed hulls lowered the *in vitro* degradation of their proteins; the addition of the PEG partly removed that effect (Yu *et al*., 1996). *In vivo* studies have also reported that the main effect of tannins is to limit protein degradability. Many researchers have reported decreasing ammonia concentration in the rumen in response to tannin utilisation (McNabb *et al*., 1993; Al-Dobaib, 2009). This has been ascribed to lesser solubility and

decreased deamination of plant proteins in the presence of tannins. This obvious decrease in protein accessibility to rumen microbes is often followed by increased post-ruminal N flow (Barry & Manley, 1984; Barry *et al*., 1986; Waghorn *et al*., 1987; 1994b; McNabb *et al*., 1996).

1.6.6.6 Effects of tannins on nutritive value

Based on ruminant nutrition, disadvantages and advantages of tannins are commonly reported in the literature. For instance, as dietary protein is first obtainable to the rumen micro-organisms than to the ruminant, precipitation of dietary proteins by tannins might result in improved efficiency of protein utilisation. In term of high quality forages, protein utilised by rumen micro-organisms may be a way of wasting protein (Mangan, 1988). In fact, problems linked with extensive proteolysis or deamination in the rumen limit production in modern feeding systems. By limiting the access of protein to rumen micro-organisms or their enzymes, tannins could enhance the escape of dietary protein to the abomasum and small intestine of ruminant fed tannin-rich forages (Abdulrazak *et al*., 2000a; Krebs *et al*., 2003). Other potential useful effects of tannins in ruminants include the avoidance of bloat in cattle (Mangan, 1988; Kumar & Vaithiyanathan, 1990; Mueller-Harvey, 2006; Waghorn, 2008) and the control of internal parasites in ruminants without the recourse to anthelmintics (Niezen *et al*., 1996; Ahmed, 2010). Tannins consumed in high quantities might decrease forage consumption, possibly due to reduced forage palatability and digestibility and by inhibition of digestive enzymes. Given the intricacies of the ruminant digestive tract, evaluation of the nutritional effects of tannins is not simple. Furthermore, tannins are only one among the wide array of secondary metabolites produced by plants. Dietary tannin reduces protein and DM digestibility in some animals (Robbins *et al*. 1987a, b) but does not affect digestion in others (Driedger & Hatfield 1972). Some-times, tannin may act as a toxin rather than an inhibitor of digestion (Mehansho *et al*. 1987). The variety of effects of tannin on digestion is due to partly to differences in the physiological abilities of animals to grip tannins and partly to differences in the chemical reactivity of various types of tannins. Mice offered diets containing tannins or saponins experienced adverse effects on voluntary feed intake and weight gain, but when mice had an option to choose between tannin and saponin containing diets, they selected mixtures that limited the ill- effects of either metabolite alone.

1.6.7 Effects of fibre and tannins on voluntary feed intake

Feed factors such as high roughage limit feed intake by animals because of physical fill of undigested feed (roughage) residues in the rumen (Madsen *et al.,* 1997). Ingested feed passage rate from the rumen may also influences the VFI of ruminants (Beever, 1993). The maximization of DM coexist in ingested feed particles in the rumen may refer to the high percentage of soluble matter, microbial digestion activity or propulsion of digesta from reticulo-rumen into the omasum (Kennedy & Doyle, 1993). Every feed factor that raises the rate of breakdown of large particles may raise the rate of passage of small particles from the rumen (Wilson & Kennedy, 1996).

High quantity of fibre negatively influence daily feed intake of ruminants thus this feed requires processing (Lu *et al*, 2005). Milling, grinding, chopping, or pelleting improve feed intake (Walker, 1984). Processing decreases by the length of fibres and limited the opportunity of animal to select among different parts of feed and therefore, increasing in feed intake and reducing the feeding time. For example, when hay is given to animal without any chopping, the animals have more opportunity to choose between stem and leaf. Feed intake is improved by 60% when browse species are chopped before offering to the livestock (Omokanye *et al*., 2001).

Structural carbohydrates (cellulose and hemicellulose) and lignin increase the fibre content of the plant. A decrease in digestibility of diet is associated with an increase in the amount of the fibrous component. Lindroth (1989) pointed out that high cellulose is associated with low digestibility. This decrease is ascribed to an increase in the mean retention time of the diet in the gastro-intestinal tract (Wilfart *et al.,* 2007), that may be due to an increased mucus secretion and water holding capacity. High fibre content may also limit the extent of energy availability as high fibre content cause an earlier satiety, although the animal's energy intake is incomplete (Wenk, 2001).

Ruminants could forage more on younger plant material than on mature ones due to an increase in fibre (cell wall) contents of mature plant. According to Chaves *et al.* (2006) this may refer to low protein content in mature plant. Legumes are rich in nitrogen and considered to improve the diet of the foraging animal (Baloyi *et al.,* 2006). The higher ratio of cell content to cell wall of legumes than in grasses make legumes comparatively more

palatable and digestible (Tainton, 2000). In contrast, it has been reported that legumes have a wide range of anti-nutritional factors such as tannins (Baloyi *et al.*, 2007).

The existence of tannins in forages influences voluntary feed intake (Barry & Duncan, 1984; Reed, 1995; Min *et al.*, 2003; Baloyi *et al.*, 2006; Alonso-Díaz *et al.*, 2008), possibly as a result of reduced palatability or limited digestion of ingested feed (Reed, 1995; Decandia *et al.*, 2008). Kumar and Vaithiyanathan (1990) suggested that a high level of tannins would reduce feed intake in three ways. Firstly, tannins may decrease the digestion of dry matter in the rumen, respond to the external cellular layer of the gut wall (Mitjavila *et al.*, 1977) and thus reduce the permeability of the gut wall. This would offer a significant feedback in the ruminant for managing the feed intake. Secondly, tannins may limit the level of some hormones (e.g. bombesin) and influence plasma growth hormone in sheep (Barry, 1984). Thirdly, the depression of intake could also be due to unpalatability of the feed (Gilboa *et al.*, 2000; Decandia *et al.*, 2000; Patra & Saxena, 2011). In contrast, a number of studies have reported that tannins do not affect feed intake (Kumar & Vaithiyanathan, 1990; Wang *et al.*, 1994; 1996a; Frutos *et al.*, 2004; Mueller-Harvey, 2006; Waghorn, 2008) in case of lower tannin concentrations (Barry & Manley, 1984; Waghorn *et al.*, 1994a; Aerts *et al.*, 1999). Other studies have reported that tannins (< 50 g kg^{-1}) reduced feed intake (Hervas *et al.*, 2003; Beauchemin *et al.*, 2007). These differences in feed intake among studies may be due to the differences in the concentrations and sources of tannins fed and the diverse methodology applied for tannin determination. Moreover, when offered as protein supplements for poor quality basal diets, legumes with high tannin contents are associated with low basal diet intake, whereas those containing moderate tannin levels are associated with higher basal diet intake (Norton & Ahn, 1997). These influences of tannins are significant when tannin-rich legumes are offered as protein supplements to crop residues (Reed, 1995; Ouda & Nsahlai, 2007). Waghorn *et al.* (1994a) assumed that reduced ruminal turnover and rate of digestion was more significant than palatability in decreasing intake of sheep fed pure *Lotus pedunculatus* in comparison to sheep fed *L. pedunculatus* plus PEG. A feature of the legumes in which tannins appeared to negatively influence intake is that *in vivo* dry matter digestibility in these legumes was low. Conversely, *in vivo* digestibility of *Lotus corniculatus* was no less than 68%. It could be probable that in ruminant when energy is limited the nutritional impact of tannins is more important.

In ruminant, there is very little information concerning the tannin-energy interplays in digestive system. In this issue, a factor that plays the main role is the dry matter digestibility which associates with fibre composition and degradability. It is known that herbivores select leaves more than stems, although leaves are extremely high in tannin content (Salawu *et al*., 1997a). Similar observations regarding leaf-stem selectivity by sheep were made by Nsahlai *et al*. (1998b). In contrast, stems had higher NDF and more indigestible ADF than leaves. Leaves are more easily cropped than stems because of the lower resistance (fibre) of leaves to chewing and concerning the stem material, it has long ruminal retention time because of their high fibre contents. In brief, estimates of the influence of CT on the voluntary intake of feeds have still not reached an acceptable point of confidence. To evaluate tannin impacts, some studies have been conducted using pure tannins or tannin-binding agents. Pure tannins added to a tannin-free diet, or tannin-binding agents added to a tannin-rich diet, both appear to be acceptable to studies of the tannin impact on intake of tannin containing forages. Results found from these last approaches are prone to be confounded by many non-tannin factors. However, no approach is free from criticism.

1.6.8 Effects of fibre and tannins on digestibilityof nutrients

The digestibility and nutrient contents of feed are a principal measure of their value to the animal as a source of energy (McDonald *et al*., 2002). Digestibility is strongly linked to the lignin contents (Jung & Allen, 1995; Moore & Jung, 2001) and fibre content (cellulose and hemicellulose) (Jung & Allen, 1995; Sanon *et al*., 2008). Digestibility is determined as the difference between the amount of feed consumed and the amount excreted as faeces. The energy measured in the excreta is not all derived from the forage, and all the energy is only partially accounted for in metabolic rate. Thus, during feeding trials, the apparent digestible energy is determined. It is known that the relationship between digestibility and feed value is positively related to the concentration of nutrients and intake of the forage. Thus, feed can be highly digested if the amount of nutrients in the feed is high (Sanon *et al*., 2008). This relation is, however, affected by factors such as the animal species, level of feed intake, and growth rate of the animal (McDonald *et al*., 2002) and interaction between plant chemical composition particularly between tannins, and protein and carbohydrate.

It has been suggested that tannins may act as defence mechanisms in plants against herbivory by reducing cell wall digestion. This may depend on the tannin (type and concentration) consumed (Robbins *et al*., 1987b; Seigler, 1998; Waghorn, 2008; Patra & Saxena, 2011) and the animal species (Robbins *et al*., 1987b). Ramírez *et al*. (2000a) and Patra and Saxena (2011) reported that dry matter digestibility for plant leaves decreased with increased tannin content.

Moreover, several studies have reported negative correlation between the dry matter and fibre digestibility, and tannin content in forages (Nuñez-Hernandez *et al*., 1991; Waghorn & Shelton, 1995; Wang *et al*., 1996a; Ben Salem *et al*., 1997b; Norton & Ahn, 1997). However, other studies have shown different results between *in vitro* and *in vivo* experiments. Accordingly, the use of *in vitro* techniques to estimate the effect of tannin-rich plants on digestibility has faced some criticisms. These criticisms are based on (i) lack of absorption and outflow of digested particles of fibrous, volatile and soluble feed constituents from the *in vitro* system compared to the rumen (ii) lack of extensive dilution *in vitro* as what happens in rumen via salivary buffer, (iii) lack of addition and continuous mixing of saliva with diets prior to and during fermentation *in vitro* compared to the rumen and (iv) lack of similarity between solubility *in vitro* and absorbability *in vivo*. However, until now, there is no alternative technique than the *in vitro* one to determine the tannins effects. Additionally, results from *in vivo* study have also recorded variable responses on fibre and dry matter digestion because of tannin consumption. For example, Barry and Duncan (1984) reported that sheep fed on tannin-rich forage had decreased apparent digestibility of fibre (cellulose and hemicellulose) compared to those fed tannin-rich forage plus PEG. Moreover, several researchers have reported decreased *in vivo* digestibility of fibre and/or dry matter due to the presence of contrasting levels of tannins in forages (Terrill *et al*., 1989; Wang *et al*., 1994; Waghorn & Shelton, 1995; Wiegand *et al*., 1995). Nuñez-Hernandez *et al*. (1991) observed high digestibility for NDF in small ruminants fed tannin-containing mahogany, as compared to those treated with PEG (2.3 g/g of tannins). Furthermore, some studies also reported that the presence of tannins was related to reduction of short chain fatty acids such as iso-butyrate, iso-valerate and n-valerate (Terrill *et al*., 1992a; Waghorn & Shelton, 1995; Wang *et al*., 1996a). The influence on rumen micro-organisms will be discussed in grater detail in section 1.6.6.4.

Studies on the impact of tannins on the digestion of lignin have demonstrated that the presence of tannins is associated with a reduction in the apparent digestibility of lignin. This is improved by adding PEG (Barry *et al*., 1986; Reed, 1986; Silanikove *et al*., 1994). In addition, ruminal digestion of lignin as a percentage of total digestibility was reduced as tannin contents increased in the diet (Barry *et al*., 1986; Reed, 1986). Silanikove *et al*. (1994) showed that *Cerutoniu siliquu* with high concentration of tannins had lower ADL digestibility by sheep. Likewise, sheep and goats fed on tannin-rich *Acacia saligna* had negative ADL digestibility (Degen *et al*., 1995). Makkar *et al*. (1997b) examined how the occurrence of tannins could interfere with the estimation of *in vitro* rumen true digestibility. The NDF and ADF determined by centrifuging the liquid phase after the incubation was compared with those determined directly. The NDF which was obtained by centrifugation was significantly higher compared to those obtained directly, while the reverse was true in the case of ADF. This was attributed to the existence of higher quantities of nitrogen and tannin in the ADF of both centrifuged and direct sets than in the ADF of the sample incubated. The same was true in the case of NDF obtained by centrifugation. These explanations suggest that the detergent system of plant analysis is insufficient for the determination of *in vivo* and *in vitro* cell wall digestibility of tanniniferous forages. The use of the detergent system for the determination of lignin *in vivo* digestibility were established by the study of Goodchild and McMeniman (1994) in an experiment planned to observe the disappearance of sorghum stover polyphenols and CT from browse in the sheep gut. The disappearance of tannins at different parts of the gut was relatively similar to the amount increasing in artefact lignin. This artefact lignin had 74 mg N per g, three times above the N content in lignin, but less than the N content in Maillard products (Van Soest, 1982); and thus, mobilised several times less N than tannin-protein complex would have done. Van Soest (1982) assumed that change of tannins in or around the abomasum into an inert lignin component would clarify why tannin changes rumen degradation more than it limits the digestion of protein in the small intestine.

1.7 Foraging on tannin-containing plants

Animals that usually use tannin-containing plant as feed should have developed at least few successful defences against the probably harmful effects of tannins. Additionally, it would be logical to assume that the protective responses observed in a given animal would be related to the nature of the problem the animal faces. The first line of defence is control

of intake. Mueller-Harvey and McAllan (1992) hypothesised that ruminants are able to minimise tannin intake in the case of free choice. This was supported by Jansen *et al.* (2007) who suggested that tannins are not avoided completely by goats but they are kept below a certain threshold. Moreover, goats could limit the intake when plant species have high concentrations of tannin and lignin (Dziba *et al.*, 2003a). However, many studies assumed that phenols and tannins strongly influence food selection by herbivores (Provenza & Malechek, 1984; Bryant *et al.*, 1991; Palo *et al.*, 1997). It should be kept in mind that diet selection is the outcome of the interaction of different factors. For example, prior experience in life might continuously affect feed intake (Distel & Provenza, 1991; Provenza, 1995; Provenza *et al.*, 2003; Provenza & Villalba, 2006). Distel and Provenza (1991) offered low quality tanniniferous shrubs to young goats that consumed more shrub than goats that had never been offered tannin-rich feeds. This confirms the findings that previous experience with better ingesta improves animal preferences to these feeds (Villalba & Provenza, 2000).

Such remarks mean that many physiological and morphological factors are actively included in these adaptive responses. This was shown in the work of Distel and Provenza (1991), where experienced and inexperienced goats were exposed a choice between older growth (low tannin) and current season growth (high tannin) of shrub, early in the study period, inexperienced goats ingested more high tannin shrub than experienced goats. However, inexperienced goats quickly (24 hours) learned to avoid selecting the high tannin shrub, assuming that CT stimulated the emetic system of the midbrain and brain stem (Provenza *et al.*, 1990). Later, both groups avoided high tannin shrubs; however, experienced animals had considerably better intakes of low tannin shrub than inexperienced animals. This better intake was associated with increased rumen capacity and possibly to a better ability to detoxify ingested phenols in experienced animals. This was confirmed by Baloyi *et al.* (2006) who suggested that the animal has to avoid foraging on plant with high content of tannin. Whereas some animals are able to detoxify, they still need to balance the rate of intake with the rate of detoxification if they are to eat successfully forages containing absorbable phenolics (Jansen *et al.*, 2007). Similarly, sheep offered high tanniniferous *Acacia* spp balanced tannin consumption, with intake altering from day to day (Ben Salem *et al.* 1997a). These results agree with the hypothesis that mammalian herbivores ingest toxins in amounts they can detoxify (Freeland & Janzen, 1974).

An additional, line of defence against ingested tannins is the production of salivary proline-rich proteins (PRP) that have very high affinity for tannins. It has been suggested that these proline-rich tannin-binding salivary proteins act as the first line of defence against tannins in the digestive tract (Mehansho *et al*., 1983). The parotid salivary glands in ruminants are four times bigger in browsers than in grazers (Robbins *et al*., 1995), which could be best explained by the range of tannin intake among these two groups of feeders. They recorded that the saliva of the browser (deer) had a higher proline content and greater tannin-binding capacity than saliva of the grazers (sheep and cows). Results from previous studies suggested that browsers that normally ingest dietary tannins produce tannin-binding salivary proteins, but the grazers do not produce such proteins (Austin *et al*., 1989). The efficacy of this adaptation was investigated by Robbins *et al*. (1991), who reported that the digestibility of fibre by deer, which secreted proline-rich proteins, was not influenced by condensed tannins, but it was reduced in sheep which have saliva with low affinity for tannins. Austin *et al*. (1989) also suggested that tannin-binding salivary proteins form soluble complexes with tannins that are stable at the pH found during passage of digesta through the whole digestive tract. Provenza and Malechek (1984) reported that salivary or plant protein utilised by goats might bind as much as 50% of the dietary tannins during ingestion. Moreover, Juntheikki (1996) reported that moose secret a salivary protein which binds only CT in their diet. It is remarkable that production of tannin-binding salivary proteins appears to be constitutive in all other animals and in amounts which reflect their approximate intake of tannins and related polyphenols (Butler, 1989). A disadvantage is that if tannin-binding proteins have lower digestibility (as is the case), its continuous secretion might lead to decreased protein retention once animals eat low-tannin diets (Robbins *et al*., 1987b). However, Mole *et al*. (1990) reported that mammalian proline-rich proteins have very low levels of nutritionally essential amino acids, which should make them expendable at a low cost for the animal. Proline is not regularly an assurance of protein having high affinity for tannins. For example, Mole *et al*. (1990) found high proline content in salivary proteins from sheep (46%) and cows (57%) that had no affinity for tannins; conversely, the lower proline in deer saliva had strongly bound tannins.

1.8 Performance of ruminants foraging on tannin-containing plants

The mechanisms and effects of tannin-ruminant interactions are so varied that precise predictions of animal performance (i.e. milk, meat or wool production) as influenced by

tannin consumption cannot be easily made. There are huge varieties of tannin-nutrients, tannin-micro-organisms and tannin-animals interactions that it would appear inaccurate to attempt to locate an explanation for these contrasting occurrences. A number of studies that report positive effects of tannin in terms of animal productivity can be found in the literature. That may be due to moderate levels of tannins, since better animal performance is associated with moderate levels of tannins in the feed which has been ascribed to the protection of dietary protein from microbe degradation in the rumen, thus an increase in flow of dietary protein to the small intestine and then an increase in the chance of the amino acid absorption into blood (Makkar, 2003; Patra & Saxena, 2011). Aerts *et al.* (1999) concluded that moderate amounts of CT (20–40 g kg^{-1} DM) in forages may exert beneficial effects on protein metabolism. Driedger and Hatfield (1972) found increased daily weight gains (DWG) in lambs fed a corn based diet supplemented with tannin-treated (10% of Tara tannin) soybean meal (DWG: 217 g) as compared to lambs receiving supplemental urea (DWG: 112 g) or soybean meal with no tannin added (DWG: 117 g). Wang *et al.* (1996b) reported that lambs grazing *L. corniculatus* obtained better wool growth and carcass gain compared to those grazing lucerne, that was attributed to the presence of CT (34 g kg^{-1}) in *Lotus corniculatus*. Terrill *et al.* (1992a) found that tannins (4.0-5.0% of dry matter) in sulla (*Hedysarum coronarium*) increased wool growth rate in sheep during spring and early summer as compared with sheep grazing the same pastures but given a daily dose of polyethylene glycol while no differences in body growth or voluntary feed intake. When treated lucerne hay with quebracho tannins 20 g kg^{-1} (equivalent to 15 g CT kg^{-1}) fed to lamb, observed increase in body weight gain and feed conversion efficiency compared with control fed untreated lucerne hay (Al-Dobaib, 2009). Moreover, when *L. corniculatus* fed to lactating ewes, milk yield was increased by 21% during mid and late lactation (weeks 6-11) (Wang *et al.*, 1996a). Similar result was found with dairy cows (milk yields (kg cow^{-1} day^{-1})) fed on *L. corniculaus* (21.24) compare to those fed on *L. corniculatus* plus PEG (to bind tannins) (18.61), ryegrass (15.53) and ryegrass plus PEG (15.49), representing that CT in *L. corniculatus* resulted in increased milk yield (Woodward *et al.*, 2000). West *et al.* (1993) reported that an increase in DM intakes, milk yield and milk fat content when lactating dairy cows fed diets containing 8–16% peanut skins (180 g CT kg^{-1}).

Tannins seem to have more advantage in the control of nematode internal parasites. Ahmed (2010) conducted two grazing experiments to evaluate dosing tannins rates for controlling

parasite in sheep. In both experiments, all dosing rates reduced the larvae counts compared to the control sheep. Ramírez-Restrepo *et al*. (2005a, b) recorded increase in body weight gain, decrease in parasite, improved reproductive performance and wool production in lambs grazing *L. corniculatus* than perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. That could be probably due to increased metabolisable protein supply, by dissociated tannin-protein complexes in the small intestine. Besides, the potential biological effects of CT on gastrointestinal parasites.

Another field that has much consideration concerning tannin-rich forages is to prevent bloat in cattle. Grazing on alfalfa and grass pastures has been frequently associated with the occurrence of bloat in cattle. The soluble proteins in these pastures are involved as surfactants responsible for the stable foams that grow in the rumen of animals suffering from bloat (Jones & Mangan, 1977). This foam captures rumen fermentation gases and the free gas space generally occur in the dorsal sac of the rumen is changed by a frothy volume of rumen digesta. Unless this condition is relieved, the animal suffers severe compression of heart and lungs, leading to anorexia and rapid death. However, forages release great amounts of soluble protein into the rumen by binding it with their content of condensed tannins (Patra & Saxena, 2011). Li *et al*. (1996) reported that most legume species are bloat-safe, since tannins in the rumen seem to reduce the solubility of protein by forming tannin-protein complexes, accordingly limiting the growing of bloat foam. In summary, it has been reported that an amount of 1.1 g CT kg^{-1} DM in the plant is sufficient to prevent the occurrence of bloat (Stockdale, 1994; Li *et al*., 1996).

A number of studies that report negative effects of tannin in terms of animal productivity can be found in the literature. Aerts *et al*. (1999) reviewed that high dietary CT contents (60–120 g kg^{-1} DM) in forages may reduce voluntary feed intakes, digestive efficiency and animal productivity. A decreased rate of body weight gain and wool growth has been reported by Barry (1985) on sheep fed *L. pedunculatus* (76–90 g CT kg^{-1} DM), which could be possibly due to the presence of high concentrations or different types of CT in forages (Waghorn & Shelton, 1997; Mueller-Harvey, 2006). Kaitho *et al*. (1998b) reported a decrease in the live weight gain of sheep with increased tannin levels, when they used six accessions of the tropical legume *Sesbania sesban*. Wiegand *et al*. (1996) reported that the high concentration of CT in *Carissa edulis* and *Dichrostachys cinerea* led to lower intake, digestibility and gain rate (6 g/day) whilst fed with maize and high weight loss (-63 g/day)

without maize. Prasad *et al*. (1997) found high wool production from sheep fed tannin-containing forage with PEG compared to those without PEG. Barry (1984) assumed that the decrease in wool production due to tannins may be for two reasons: firstly, reduced protein absorption, which restricts the quantity of sulphur amino acids accessible for wool growth; secondly, an increase of plasma growth hormone concentration which would divert amino acid away from wool synthesis. However, Wang *et al*. (1994) appeared to suggest that tannins increase the availability of sulphur for body synthetic reactions. Similarly, other researchers have reported no changes in the levels of growth hormone because of tannins (Waghorn *et al*., 1994b; Wang *et al*., 1996a). Grainger *et al*. (2009) reported that dosed daily with 163–326 g CT from *Acacia mearnsii* to dairy cows led to decrease in milk yield (from 33.0 to 31.8-29.8 kg cow^{-1} day^{-1}), fat and protein percentage of milk as well as reduced feed intake and digestibility. Certainly, a better understanding of tannin effects in ruminants is required to offer opportunity to make adequate use of the property of tannins in improving animal production.

1.9 Conclusions

An understanding of diet selection patterns seems to be a key for natural pasture management which is the basis of sustainable animal productivity in many societies, but has had less focus. Attention to natural pasture management is gaining global importance due to the increasing need to optimize utilization of scarce resources, nutrient management, reduce natural pasture area, environmental conservation and sustainability. The increasing interest in natural pasture accentuates a need for improved veld management. Administration of livestock populations depends largely on perceptive and predictive analysis of their habitat preferences, and the diet of the specific animal species, including species of plants eaten. An understanding of preferred plant species by particular livestock species is important to be able to judge which species may be introduced in a certain pasture. Derived from the plant species range and information about the palatability of the forages, the browsing capacity, and veld condition-management strategies can be developed to allow a reliable selection of suitable plant species for animal forage.

Tropical browse species constitute a good alternative feed for improving the productivity of tropical animal agriculture. However, the value of these browse as animal feed seems to be restricted by some factors, among which, the occurrence of tannins appears to play a

very important factor. Intake of condensed tannins by ruminants has been related to both positive and negative effects. Positive effects include protein protection from excessive fermentation in the rumen, potentially increasing protein absorption and preventing bloat development. Negative effects such as reduced voluntary feed intake and decreased fibre and dry matter digestibility occur because of impaired rumen function. Which of these effects is noticed in a given condition appears to be a function of factors such as the structure of tannins and their concentration in plant tissue. Determination of the function provided by these factors in evaluating the tannin impact on ruminant nutrition is important to provide information for animal productivity.

It is indisputable that great steps have been taken in our knowledge of tannin chemistry and in our understanding of the mechanisms essential to the interactions between tannins and other molecules under controlled conditions. Information has also been collected concerning the effects of tannin consumption on the nutritional status of ruminants. Nevertheless, it is also obvious that we are far from final understanding of the role of tannins with regard to animal performance. In fact, in almost all of the areas of research discussed, the field remains open and ripe for further development and inquiry. However, our ability to pursue scientific interests is today greater than ever before. A good result of this may be our ability to attempt to manipulate the effect of tannins at least in a laboratory. Tannin effects can be physically (drying) (Terrill *et al.,* 1989; Ben Salem *et al.,* 2005b) or chemically manipulated, including the use of ash (Ben Salem *et al.,* 2005b), urea (Habib *et al*., 2008), PVP, and PEG (Makkar *et al*., 1995a). Moreover, our ability to use the biological (*in vitro* and *in sacco*) techniques to determine and rank tannin-containing forages. These techniques could provide meaningful means to evaluate ruminant feeds since microorganisms and enzymes involved are sensitive to factors influencing digestion, which cannot be determined through chemical composition methods.

In this study, diet selection of browse species in subtropical savannah was determined by direct observation and an attempt to predict it by the nutritive value measurements was applied. The nutritive value of the most browse species selected by goats was determined by chemical composition, *in vitro* nutritive value measurements, *in sacco* measurements and the relationships among the measurements. The broad objective of this study was to determine the patterns of diet selection of goats on pasture woody species in sub-humid

subtropical savannah, South Africa and predict the diet selection using chemical composition, *in vitro* gas production, *in vitro* degradability and *in sacco* degradability.

Chapter 2

Diet selection by Nguni goats in the Zululand Thornveld*

Abstract

The objective of the study was to determine diet selection of goats grazing/browsing in Zululand Thornveld. An experiment was done in the early wet season (November 2007) and the late wet season (March/April 2008) at Owen Sitole College of Agriculture, KwaZulu-Natal. Diet selection was estimated using direct observation of animals and an indirect plant-based method. The browse species observed in the plant-based methods were: *Gymnosporia senegalensis* (Gs), *Acacia nilotica* (An), *Acacia karroo* (Ak), *Scutia myrtina* (Sm) and *Dichrostachys cinerea* (Dc). Selection was determined by observing at regular intervals 40 marked branches on 10 plants of each browse species. Direct observation of diet selection was done by observing two goats in a base herd of 24 castrated males when they were allowed to forage together in a paddock of 1ha. Observations were made every minute for 3 hours in the morning and 1.5 hours in the afternoon. The relative abundance of the browse species in the veld was determined and used to estimate the selection index (utilization relative to availability). In both seasons, the three most selected species according to the plant-based observations were Sm, Ak and Dc. Consistently, An experienced moderate defoliation and Gs the least. However, on the basis of the selection index, the species followed the order: An > Dc > Ak > Sm > Gs in the early wet season and An > Sm > Dc > Ak > Gs in the late wet season. The selection index was negatively correlated to all fibre attributes although the correlation attained significance ($P<0.05$) only for NDF, ADF and lignin but not for tannins and cellulose. Both methods did not rank species in the same order, thus suggesting the need for further methodological refinement.

Keywords: Browse, forage, herbivore, savannah

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2.1 Introduction

In Africa, natural vegetation makes up a major part of the diet of ruminant livestock. To enhance livestock production, it is imperative to improve pastures, comprising of grass and browse species. The improvement of such pastures is impossible to achieve without adequate understanding of browse-browser interaction and feeding behaviour. Bergström (1992) suggested active interaction between browsers and woody plants, in those woody plants respond to browsing and impact on feeding behaviour. Browsers have been shown to choose plants with high growth rates over plants with slow growth rates (Danell *et al.*, 1985). Animals have been shown to be able to distinguish between plant types and their parts (Hodgson, 1986). Biochemical and nutritive characteristics of feed provide the option to select the diet which satisfies the goats' requirements (Fedele *et al.*, 2002). Digestibility is influenced by plant fibre (Jung & Allen, 1995) whilst tannin may control diet selection and food intake since it creates unacceptable taste or reduces nutrient (protein) availability in the gastrointestinal tract (Arnold *et al.*, 1980; Robbins *et al.*, 1987a). Montossi *et al.* (1997) in New Zealand reported that a low content of condensed tannin (< 0.2%) did not influence lamb performance. Goats' feeding behaviour depends on the associative effects of diet items rather than their specific nutritive value, since goats maximize and balance intake without incurring digestive disorders (Fedele *et al.*, 2002). The objective of this study was to determine the diet selection of goats grazing/browsing in Zululand Thornveld. To achieve this objective, we tested the following hypotheses. Seasonal changes in availability and quality of forage in sub-humid subtropical savannah affect diet selection (Fourie et al., 1986; Abate, 1996). Natural differences in leaf chemistry among different plant species influence diet selection (Scogings *et al.*, 2004).

2.2 Material and methods

2.2.1 Study area

Two experiments were conducted during the early wet season (November 2007) and the late wet season (March/April 2008) seasons at the Owen Sitole College of Agriculture (OSCA), Empangeni, South Africa. Owen Sitole College of Agriculture is located at 28˚57'45"-28˚57'22"S latitude and 31˚55'31"-31˚57'22"E longitude within the Coastal Forest and Thornveld (Van Der Linden *et al.*, 2005). The mean annual rainfall of OSCA is

995-1022 mm, with a third of it falling in mid to late summer (Van Der Linden *et al.*, 2005). The study area was located north of the main gate of OSCA. Two paddocks (1 ha/experiment) were used. The paddocks were fenced and a pen (9 m x 6 m) was erected in one corner of the paddock for holding animals at night, which was equipped with one water trough.

2.2.2 Experimental animal and plant species

Twenty-four castrated male Nguni goats were used. The study focused on five dominant browse species: *Gymnosporia senegalensis, Acacia nilotica, Acacia karroo, Scutia myrtina* and *Dichrostachys cinera* (Table 2.1). The browse species were randomly sampled by collecting leaves 1.0-1.5 m above ground from three trees per species. Samples were kept in paper bags and air-dried prior to oven-drying at 60 ˚C for 48 hrs. The leaves were ground through a 1mm mesh sieve and stored in plastic bottles.

Table 2.1 Some morphological features of the five browse species

Browse species	Height (m)	Leaves	Flowers	Thorns
G. senegalensis	4-7	slightly big	Creamy - green	Sharp spines
A. nilotica	3-7	small (up to 0.4 cm)	Yellow	Curving backward, white
A. karroo	4-7	Small (0.4 - 1 cm)	Golden yellow	Straight, white
S. myrtina	2-15	Slightly big	Greenish white	Hooked, sharp
D. cinera	2-7	small (up to 0.5 cm)	Pink & yellow	Woody, straight

2.2.3 The relative abundance of woody species

For estimating relative abundance of woody species, the belt transect method was used. Transects of 2 m width, oriented north-south, were systematically placed 20 m apart. Each transect started and ended 10m from the sides of the paddock. Transects were surveyed in 20 m lengths. About 5% of the paddock area was sampled. In sampled transects, woody plants were identified, the height of all woody plants was measured and the proportion with browseable material below 1.5 m height estimated. The relative abundance of each species was calculated as the number of plants of a species divided by the total number of

plants of all species x 100. The density of each species (plants/m^2) was calculated as the number of plants of a species divided by the area of paddock.

2.2.4 Diet selection and selection index

An indirect plant-based method of diet selection involved marking 40 branches (on 10 plants) per species. Every two days, all branches were inspected and scored as browsed or not browsed. The cumulative proportion of marked branches was calculated and plotted against days. An animal-based method (direct method) was also used to determine diet selection. During foraging, two goats were selected randomly from the flock during each day of recording (different goats for each day) and closely observed continuously by two people. The selected browse species under study were recorded every minute. Browsing activity on species not being studied, and grazing activity, was recorded as ‚other browse' and ‚grazing', respectively. Observations were made for 3 hrs in the morning and 1.5 h in the afternoon on alternate days for a total of 6 days. Selection index (SI; utilization relative to availability) was estimated as: $SI_y=(C_y/D_s)/RA_y$, where SI_y is the selection index for species ‚y', C_y is the number of times species ‚y' was consumed, D_s is the sum of all observations of all species in the diet, and RA_y is the relative abundance of species ‚y'.

2.2.5 Chemicals analysis

Crude protein was determined using the AOAC (1997) method 990.03 (LECO. FP2000, Nitrogen analyzer). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using the methods of Van Soest *et al.* (1991) applied to ANKOM Technology Technique. Condensed tannin (CT) was determined according to the method described by Porter *et al.* (1986) and Makkar (1995) (Appendix 3).

2.2.6 Statistical analysis

The diet proportion, diet selection index and relative abundance were analyzed using the GLM procedure of SAS program (2002). Correlations between selection index and chemical properties were determined.

2.3 Results

2.3.1 Diet selection

In both seasons, the three most selected species according to the plant-based observations were *S. myrtina*, *A. karroo* and *D. cinerea*. Consistently, *A. nilotica* experienced moderate defoliation and *G. senegalensis* the least (Figure 2.1). However, on the basis of the selection index the species followed the order: *A. nilotica* > *D. cinerea* > *A. karroo* > *S. myrtina* > *G. senegalensis* in the early wet season and *A. nilotica* > *S. myrtina* > *D. cinerea* > *A. karroo* > *G. senegalensis* in the late wet season (Table 2.2).

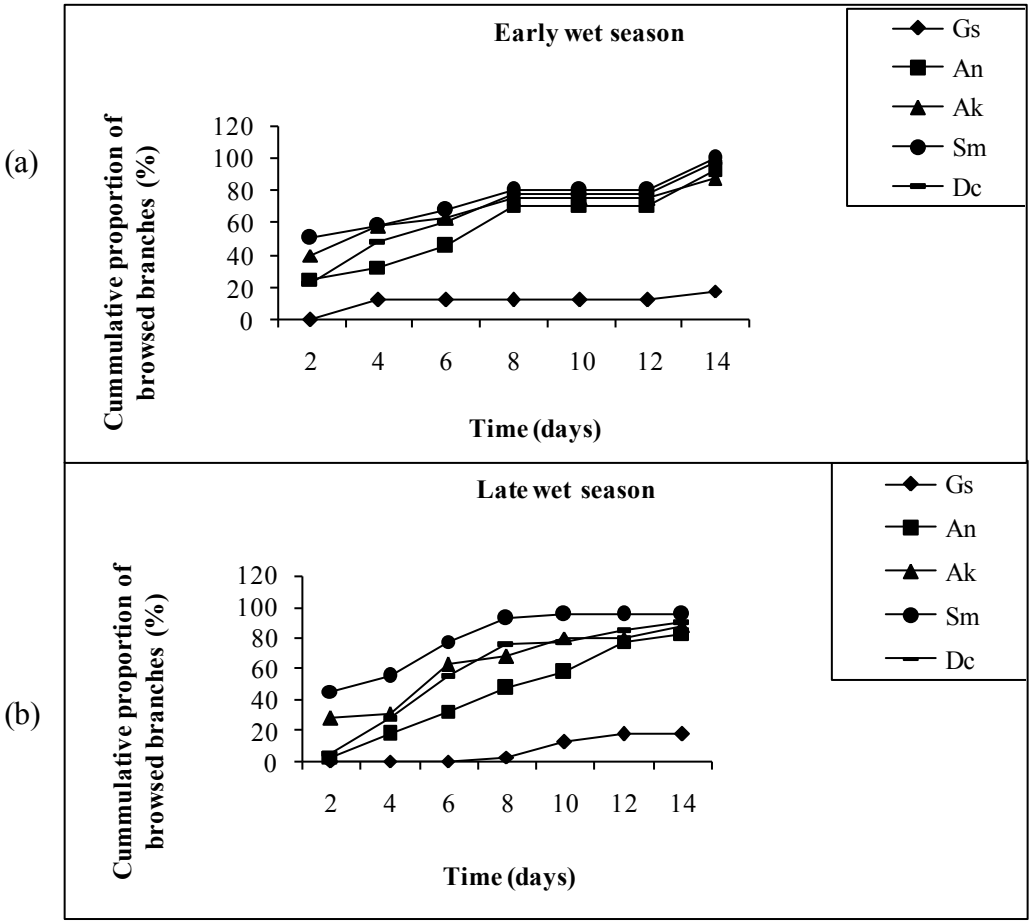

Figure 2.1 Defoliation rates of branches of browse species in (a) early wet season and (b) late wet season. Gs - G. senegalensis; An - A. nilotica; Ak - A. karroo; Sm - S. myrtina; Dc - D. cinera.

Table 2.2 Density of forage trees, browseable trees within species (BAS), relative abundance of browseable species (RAS), relative abundance of a browseable species in the diet (Diet), and diet selection index (Sindex)

Feed	Density (plants/ha)		BAS (plants/ha)		RAS		Diet		Sindex	
	Early wet	Late wet	Early wet	Late wet	Early wet	Late wet	Early wet	Late wet	Early wet	Late wet
G. senegalensis	949	1280	939	1248	0.077	0.090	0.005	0.014	0.10	0.27
A. nilotica	653	500	520	360	0.042	0.026	0.116	0.102	4.08	6.35
A. karroo	2439	1860	2224	1840	0.181	0.133	0.136	0.099	1.18	1.19
S. myrtina	1071	860	908	820	0.074	0.059	0.034	0.125	0.68	3.25
D. cinerea	898	1460	867	1361	0.071	0.099	0.065	0.083	1.40	1.36
Other browses	6936	8740	6804	8172	0.555	0.592	0.306	0.201	0.82	0.55
Grazing							0.338	0.374	-	-
RMSE							0.0607	0.027	0.55	1.13
P<							0.05	0.05	0.05	0.05

OB – other browse species; RMSE is the root mean square error; P – statistical significant level.

2.3.2 Correlation between chemical composition and selection index

The chemical composition of the leaves of the five palnts is shown in Table 2.3. The selection index was negatively correlated to all fibre attributes although the correlation attained significance ($P<0.05$) only for NDF, ADF and lignin (Table 2.3).

Table 2.3 Chemical composition of experimental species and correlation coefficients with diet selection index

Species	CP (g/kg)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	CT (g/kg)	Cell (g/kg)
G. senegalensis	73.8	486.4	304.8	205.3	77.5	99.5
A. nilotica	114.7	460.7	259.8	172.8	132.3	87.0
A. karroo	123.4	272.9	139.3	78.3	20.3	61.0
S. myrtina	125.7	431.4	288.0	213.2	100.0	74.8
D. cinerea	123.2	523.6	316.9	198.1	57.9	118.8
Correlation with selection index						
Early wet	0.56 ^{ns}	-0.88*	-0.88*	-0.90*	-0.76^{ns}	0.62 ^{ns}
Late wet	0.48 ^{ns}	-0.87*	-0.96*	-0.97*	-0.46 ^{ns}	0.69 ^{ns}

CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre; CT - condensed tannin; Cell – cellulose; ^{ns} ($P>0.05$); * ($P<0.05$).

2.4 Discussion

The three most selected species (*S. myrtina*, *A. karroo* and *D. cinerea*) according to the plant-based observations had the same ranking order in both seasons. These results supported by studies in the Eastern Cape which obtained the same order of preference for *S. myrtina* (Dziba *et al.,* 2003a) and *A. karroo* (Mapuma *et al.,* 1996; Raats *et al.,* 1996; Dziba *et al.,* 2003a). Yayneshet *et al*., (2008) showed that goats preferred *D. cinerea*. Moreover, *D. cinerea* is important and identified as likely main plant species for goats feed in most environment (Mlambo *et al*., 2004). In contrast, *D. cinerea* was not browsed by goats in West Africa savannah (Ouédraogo-Koné *et al.,* 2006; Sanon *et al*., 2007). These differences could be due to the difference of quality vegetation which could be based on differences in climate and soil of the research sites and plant composition. Sanon *et al.*

(2007) and Mkhize (2008) mentioned the lowest intake of *G. senegalensis* which is in agreement with the findings of this study. Consistently, *A. nilotica* experienced moderate defoliation. Jansen *et al*. (2007) reported that *A. nilotica* likely to be avoided by goats.

However, the ranking of plant species which are based on the selection index were varied in both seasons. *A. nilotica* and *S. myrtina* registered different indices in both season, but differences were much wider in the early wet than in the late wet season. This suggests that preference ranking might be affected by season (Dziba *et al.*, 2003a). Milne *et al.* (1979) found that the intake rate of grazing sheep differed between summer and autumn. Goats might avoid a season's twigs as a result of their high tannin contents and their location within the plant canopy (Provenza & Malechek, 1984). The similar selection indices of *A. karroo* and *D. cinerea* in the early and late wet season (1.18 and 1.4; 1.19 and 1.36, respectively) perhaps because both plants had green leaves in both seasons.

Selection of diet items may sometimes be a function of quality and other times a function of quantity (Shipley *et al*., 1999). This study found negative relation between selection and fibre attributes. These findings are supported by Moore and Jung (2001) who found a negative relationship between the fibre attributes and the digestibility of browse. Ndlovu and Nherera (1997) reported that fibre could be more important than tannins when managing fermentation *in vitro*. Arnold (1981) suggested that herbivores select diets based on the concentration of nutrients relative to toxins rather than on availability.

2.5 Conclusions

Indirect plant-based and direct animal-based methods did not rank browse species in the same order, thus suggesting the need for further methodological refinement. Diet selection appears to be influenced by the fibre constituents.

Chapter 3

Diet selection of Nguni goats in relation to season, chemistry and physical properties of browse in sub-humid subtropical savannah*

Abstract

This study was conducted to determine the influence of plant chemical, physical and phenology properties on diet selection of Nguni goats during the dry, early wet and late wet seasons in savannah in South Africa. Diet composition was estimated by direct observation of two different adult Nguni goats randomly selected from a herd each day for 7-8 days in each season. Observations were made during active foraging periods for 2 hrs in the morning and 1.5 hrs in the afternoon. The duration of each feeding bout and the species of woody plant from which bites were cropped at each feeding station were recorded. Diet selection was determined from the relative duration of feeding. Diet preference of each species was expressed as an index calculated using the selection and relative abundance of woody species. Browse species consumed by goats were sampled and analysed to determine crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, condensed tannins, cellulose and hemicellulose. Diet selection varied among the three seasons. The five species most selected (utilised) by goats were *Scutia myrtina, Acacia nilotica, Dichrostachys cinerea, Acacia natalitia* and *Chromolaena odorata*. *Scutia myrtina* was the most selected species during the dry season while *D. cinerea* was the most selected in the wet seasons. *Scutia myrtina* was the most preferred (highest utilisation relative to availability) in the dry and early wet seasons while *A. nilotica* was most preferred in the late wet season. Spinescent species were generally selected more than non-spinescent species in all seasons, while fine-leaf and deciduous species were selected more than broad-leaf and evergreen ones in the wet seasons. However, preference for broad-leaf and evergreen species increased in the early wet season. Although plant chemistry varied across seasons, it did not explain the preference of goats for various plant species in this study. Instead, effects of chemistry were species-

* Basha, N.A.D., *et al.*, (2012), Diet selection of Nguni goats in relation to season, chemistry and physical properties of browse in sub-humid subtropical savanna. Small Ruminant Res. 102, 163-171.. online: http://dx.doi.org/10.1016/j.smallrumres.2011.08.002

specific. In conclusion, this study demonstrates the importance of evergreen browse species as a source of fodder when deciduous species are unavailable.

Keywords: forage; herbivore; tannins; nutrients; leaf morphology; phenology; spinescence

3.1 Introduction

Savannahs occupy 65% of the total area of Africa, 60% of sub-Sahara Africa (Scholes & Archer, 1997) and 54% of KwaZulu-Natal province in South Africa (Breebaart *et al.*, 2002). Savannah is defined as vegetation covered by 10-50% woody plants and a well-developed grass layer. Savannah browse adds significantly to the total forage of livestock and wildlife in Africa (Bergström, 1992). Therefore, the savannah is important to support human populations in Africa through supporting livestock production (Scholes & Archer, 1997). However, African savannahs have been modified by human activities such as cutting trees for livestock grazing, wood harvesting and crop production, or by plant invasions caused by fire suppression, reduction of wild ungulates and overgrazing by domestic livestock (Scholes & Archer, 1997).

A key to improving the management of savannahs is the development of a sound understanding of diet selection. Diet selection describes the decisions animals make with regard to the plant material (plant parts, plant species and patches) they choose (Newman *et al.*, 1995). Animals frequently select and eat some species more than others (Morrison *et al.,* 2002). Furthermore, diet selection is a central key to understanding plant-herbivore interactions which is an important requirement for evaluating the impact of herbivores on environments. However, the patterns of diet selection are not regular in space or time. That may be due to changes in forage availability (Edenius *et al.*, 2002) and changeable chemistry. Diet selection patterns in goats are also controlled by variation in features among and within plant species. Furthermore, diet selection of herbivores is influenced by many factors, of which the main one is variation in the seasonality of African savannahs which leads to differences in forage composition and availability (Dziba *et al.*, 2003a). Additionally, differences in chemical and physical defence have influenced the diet selection by herbivores (Provenza & Balph, 1990; Illius *et al.*, 1999).

Chemicals such as tannins are assumed to function as defences against herbivores (Bergström, 1992). Besides changing the taste of feed, the negative feedback of tannins causes rumen microbial inhibition, decreased digestibility and animal performance (Silanikove *et al.*, 2001; Min *et al.*, 2003; Mueller-Harvey, 2006), but is advantageous in goats in dealing with tannin diet and that tolerate them without exhibiting toxic syndrome (Silanikove *et al.*, 1996; Landau *et al.*, 2000; Silanikove, 2000). However, tannins are not totally avoided by goats but are tolerated at a certain minimum level (Jansen *et al.*, 2007). It has been suggested that the minimum level of tannin for ruminants to tolerate in forages is 55 g/kg DM (Min *et al.*, 2003), but if this amount is exceeded and free tannins reach the rumen, tannins can form indigestible complexes with bacterial enzymes (Barry & Manley, 1986; Priolo *et al.*, 2000; Khanbabaee & van Ree, 2001), or cellulose and hemicelluloses (Haslam, 1989). Thus, tannins can protect plant cell walls against rumen organisms (Jachman, 1989) by attaching to the cellulose and fibre-bound proteins of the cell walls. These result in reduced animal performance (Priolo *et al.*, 2000). In spite of these negative effects, tannins have many other positive effects on ruminant animals such as increased nitrogen retention when protein-tannin complexes dissociate in the post-rumen (Nsahlai *et al.*, 1998a; Mueller-Harvey, 2006). This may provide additional amino acids for digestion, absorption and utilization by the animal (Karchi, 1998; Min *et al.*, 2003) and it prevents excessive degradation of high-quality leaf protein in the rumen (Mehansho *et al.*, 1987).

Ruminants need sufficient dietary fibre for standard rumen functions. This is related to maintaining enough saliva and optimal pH for cellulolytic microorganisms that produce short chain fatty acids (SCFA) in the rumen liquor (Church, 1988). Fibre has a significant role in goat production because of its influence on the intake and digestion of nutrients (Lu *et al.*, 2005). Through their control of mastication, fibre limits intake by reducing the intake time and maintains normal fermentation in the rumen by introducing saliva (Lu *et al.*, 2005). Hence, fibre fractions of browse could be significant for fermentation (Ndlovu & Nherera, 1997). Minimizing acid detergent fibre is a good way to explain preferences in the case of *Acacia* species (Jansen *et al.*, 2007). Moore and Jung (2001) reported a negative relationship in general among the fibre fractions, particularly lignin, and the digestibility of browse. Therefore, the challenge is to understand the relationship between fibre and diet selection by goats, corresponding to the change of fibre fractions throughout the year.

In addition to plant chemistry, diet selection in mammalian herbivores can be influenced by physical plant factors such as spines and thorns. Physical features such as thorns and spines; they may limit leaf accessibility and intake rates resulting in lower preference of species that have these traits by browsers (Haschick & Kerley, 1997b; Dziba *et al*., 2003a). Wilson and Kerley (2003b) reported that spinecence affects foraging efficiency of goats. Additionally, the effect of spinescence depends on the body size of herbivores (Cooper & Owen-Smith, 1986). Shipley *et al.* (1998) found that diet selection by moose is related to plant morphology.

The objectives of this study were (i) to explore the diet selection of goats foraging in different seasons in a sub-humid subtropical savannah, and (ii) to investigate the possible relationships between diet selection and various physical, chemical and phenological features, such as condensed tannins, crude protein, fibre fractions, spinescence, leaf type and deciduousness. To achieve these objectives, we tested the following hypotheses. Firstly, seasonal variations in availability and quality of forage in sub-humid subtropical savannah alter diet selection (Abate, 1996). Second, inherent differences in leaf chemistry among different plant species affect diet selection (Scogings *et al.*, 2004). Thirdly, variations of different plant species in leaf morphology (Shipley *et al.*, 1998; Dziba *et al.*, 2003a, Wilson & Kerley, 2003a), spinescence (Cooper & Owen-Smith, 1986; Gowda, 1996) and leaf phenology (Shipley *et al.*, 1998; Watson & Owen-Smith, 2002) influence diet selection.

3.2 Materials and methods

3.2.1 Study area

The study was conducted at the Owen Sitole College of Agriculture (OSCA) in northern KwaZulu-Natal, South Africa. OSCA is placed within the Zululand Coastal Forest and Thornveld (Mucina & Rutherford, 2006) and is located at 28˚57’45”-28˚57’22”S latitude and 31˚55’31”-31˚57’22”E longitude (Van Der Linden *et al.*, 2005). The mean annual rainfall of OSCA is 995–1022 mm, with 75% of it falling in the wet season (October – April). The experimental paddocks (2-2.5 ha each) were fenced and a pen was erected in one corner of each paddock to keep the animals at night. Confining livestock in enclosures at night is commonly practiced in the area to prevent theft and predation. Ad lib water was

provided in a trough. Fieldwork was carried out during the dry season (late June/early July, 2008), early wet season (late November/early December, 2008) and late wet season (late February/early March, 2009).

3.2.2 Relative abundance of woody species

For estimating the relative abundance percentage of woody species, the vegetation was recorded by means of 2 m wide belt transects oriented north-south. Each transect started and ended 10 m from the edges of the paddocks. Impenetrable thickets of *Chromolaena odorata* were avoided. Because each paddock was not quadrangular, transect lengths varied between 20 and 120 m, but ultimately 5% of each paddock area was sampled. In each transect, the height (cm) of each woody plant was measured and identified to species level (according to Coates Palgrave, 2002). When there was no foliage below 1.5 m the plant was recorded as unbrowseable. The data from all transects in a paddock were used to calculate the relative abundance of each species, which was calculated as the total number of browseable plants of each species divided by the total number of browseable plants of all species.

3.2.3 Species samples and chemicals analysis

Browse species and grass which were eaten by goats were sampled. Species that were avoided by goats were not sampled because the study focused only on species utilized by goats during the three seasons of the study. The browse samples were taken from three unbrowsed trees per species and grasses were cut from three different ungrazed areas. Plant samples were collected in the last two days of each period, kept in paper bags and air dried prior to oven drying at 60 °C for 48 hours. Samples were ground through 1 mm mesh and analyzed for chemical composition on a dry matter basis. Crude protein (CP) was calculated using nitrogen concentration which was determined by AOAC method 990.03 (AOAC, 1997) using a LECO, FP2000, nitrogen analyzer. Nitrogen was converted to CP using 6.25 as a conversion factor. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991) using the ANKOM Technology Technique. The acid-butanol proanthocyanidin assay (Porter *et al.*, 1985) was used to determine condensed tannins (CT) (Makkar, 1995)

(Appendix 3). Cellulose was calculated as the difference between ADF and ADL, while hemicellulose was derived from the difference between NDF and ADF.

3.2.4 Diet selection and selection index

Previous browsing influence on chemical traits (protein and tannin) for the main species (*Scutia myrtina, Acacia nilotica, Dichrostachys cinerea, Acacia natalitia*) was tested because one paddock was used twice. Samples from ten trees per species per area were sampled and analyzed for protein and condensed tannin. Control samples were taken from an area that was not previously browsed. Previous browsing did not influence the protein (t = 0.46, df = 118, P = 0.32) and tannin contents (t = -1.34, df = 118, P = 0.092) compared to unbrowsed species. Diet selection was estimated over seven or eight consecutive days during each season by direct observation of goats while feeding. Observations were made between 08:30 and 10:30 and from 12:00 onwards until observations had been collected for at least 1.5 hrs. Each day, two different goats were randomly selected from a herd of 22-24 castrated males that were 3 years old and weighed avarage 45.8 ± 0.78kg (mean ± SEM:). The two selected goats were allowed to forage with the others and each goat was continuously observed from a distance of about 5 m by two people, with minimal disturbance. The flock was herded to a new, randomly selected part of the paddock at the start of each day so that depletion of forage would not be a factor in diet selection. The size of the paddock was big enough to allow us to do this.

Feeding bouts were defined as any event 5 seconds (s) long or longer that the animal spent biting material from a plant. Events of biting that were less than 5s were ignored. The duration of each feeding bout and the species of woody plant from which bites were cropped were recorded. Grazing bouts were simply recorded as grazing (without identifying grass species). When a focal animal was lost from view for 5s or more, the period of time for which it was not visible was recorded. The diet selection index (SI) of each species which was eaten by goats was calculated. Species on which goats spent very little time were summed together as "other browse" species (OB). Diet selection was estimated according to relative duration (total time spent on a species/total time spent on all species) rather than frequencies of bouts due to large variation among bout durations. Selection index was calculated according to Ivlev's forage ratio (E_i) using the relative abundance of the species in the diet divided by the relative abundance of the species in the

available vegetation, because this method is simple, yet does not produce results different to more complex methods (Tanentzap *et al.*, 2009). Selection index values above 1.0 indicate preference while values less than 1.0 indicate avoidance. Potential seasonal variations in feed availability were assumed to be negligible because of the humid climate of the study area.

3.2.5 Statistical analysis

Analysis of variance was used to test effects of season and plant species on diet selection and selection index, and separation of means was done using the probability of difference (SAS, 2002). Correlation was used to test the relationships between selection index and chemical variables. The effects of spinescence (spines vs no spines), leaf type (broad vs fine), or phenology (evergreen vs deciduous), or combinations thereof on selection index, were analysed using t-tests. Goats were regarded as replicates. Significant effects were stated at the probability level of $P < 0.05$.

3.3 Results

3.3.1 Diet selection

Goats spent 28, 38 and 22% of their time grazing during the dry, early wet and late wet seasons, respectively. Goats selected the following species in the three seasons: *Scutia myrtina, Acacia nilotica, Dichrostachys cinerea, Acacia natalitia* and *C. odorata* (Table 3.1). The selection of plant species depended on the season of observation ($F_{17, 396} = 7.11$, $P < 0.001$). In the dry season, *S. myrtina* was the most selected, followed by *C. odorata, A. natalitia, D. cinerea* and *A. nilotica*. During the early wet season *D. cinerea* was most selected, followed by *A. natalitia, S. myrtina, C. odorata* and *A. nilotica*, while in the late wet season, *D. cinerea* was most selected followed by *A. nilotica, S. myrtina, A. natalitia* and *C. odorata*.

The species included in the diet covered a range of physical traits or phenology (Table 3.2). Spinescent species were selected more than spineless species ($t = -9.20$, $df = 271$, $P < 0.001$), while fine-leaf species were selected more than broad-leaf species ($t = -4.66$, $df = 301$, $P < 0.001$) (Figure 3.1). Furthermore, deciduous species were selected more than

Table 3.1 Effect of season and plant species on diet compositions for goats (mean ± SE)

Species	Season		
	Dry	Early wet	Late wet
Acacia natalitia	0.089 ± 0.013	0.101 ± 0.011	0.098 ± 0.013
Acacia nilotica	0.061 ± 0.015	0.050 ± 0.008	0.124 ± 0.019
Chromolaena odorata	0.119 ± 0.015	0.051 ± 0.013	0.084 ± 0.011
Dichrostachys cinerea	0.069 ± 0.010	0.142 ± 0.016	0.205 ± 0.024
Scutia myrtina	0.223 ± 0.040	0.083 ± 0.020	0.118 ± 0.026
Lantana camara	0.021 ± 0.004	-	0.015 ± 0.005
Other browse	0.089 ± 0.014	0.151 ± 0.026	0.088 ± 0.012
Psidium guajava	0.006 ± 0.002	-	-
Pavetta lanceolata	0.029 ± 0.007	-	-
Rhus pentheri	0.022 ± 0.007	-	-
Jasminum multipartitum	-	0.040 ± 0.006	0.039 ± 0.010
Coddia rudis	-	-	0.015 ± 0.005

Table 3.2 Life form, leaf morphology, spinescence and phenology of major plant species frequently browsed by goats in Zululand Coastal Thornveld

Plant species	Life form	Leaf morphology	Spine	Phenology
Acacia natalitia[a]	Tree	fine	√	deciduous
Acacia nilotica	Tree	fine	√	deciduous
Chromolaena odorata[b]	Climber	broad	×	evergreen
Dichrostachys cinerea	Tree	fine	√	deciduous
Scutia myrtina	Shrub	broad	√	evergreen
Coddia rudis	Shrub	broad	×	evergreen
Lantana camara	Shrub	broad	×	evergreen
Psidium guajava	Tree	broad	×	evergreen
Pavetta lanceolata	Shrub	broad	×	evergreen
Rhus pentheri	Shrub	broad	×	evergreen
Jasminum multipartitum	Climber	broad	×	evergreen

[a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002).
[b] Invasive non-native species.

evergreen species (t = 3.79, df = 294, P < 0.001) (Figure 3.1). When the data were analysed within seasons, the proportion of spinescent species in each season was significantly higher than that of spineless species (Table 3.3). Goats included a larger amount of fine-leaf species than broad-leaf species during the early and late wet season (Table 3.3). Likewise, deciduous species comprised a larger proportion of the diet than evergreen species during the early and late wet seasons (Table 3.3).

3.3.2 Selection index

The most abundant species were also the most preferred in the three seasons, which were *S. myrtina, A. nilotica, D. cinerea, A. natalitia* and *C. ordata* (Table 3.4 and 3.5). Selection index of species depended on season of observation ($F_{13,324}$ = 4.48, P < 0.001). *Scutia myrtina* had the greatest selection index in the dry season, followed by *Rhus pentheri, Lantana camara* and *A. nilotica*. In the early wet season, *S. myrtina* had the highest index, followed by *Jasminum multipartitum, D. cinerea* and *A. nilotica,* while in the late wet season *A. nilotica, L. camara, S. myrtina* and *D. cinerea* were most preferred.

When the data set was pooled according to physical traits or phenology within seasons, fine-leaf species had a higher mean selection index than broad-leaf species in the dry season (t = 1.16, df = 129, P < 0.029), while in the early wet season the opposite was observed (t = 3.28, df = 83, P < 0.001) (Table 3.6). Goats preferred spinescent species more than spineless species in the dry and early wet seasons (t = -4.65, df = 102, P < 0.001 and t = -3.91, df = 78, P < 0.001, respectively) (Table 3.6). Only in the early wet season, evergreen species had a significantly higher selection index than deciduous (t = -3.28, df = 253, P = 0. 002) (Table 3.6). However, when the data were pooled across the seasons, only selection index of spinescent species was significantly higher than those of spineless species (2.06 ± 0.153 vs 1.26 ± 0.144, t = -3.78, df = 316, P < 0.001).

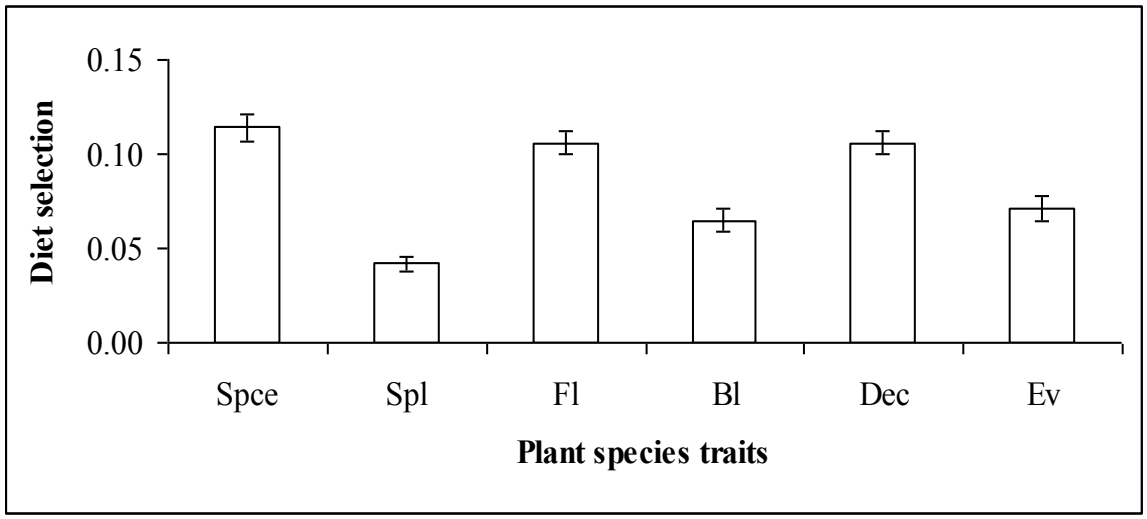

Figure 3.1 The effect of physical traits and phenology of plant species on diet selection (proportion) across the seasons (the data across 22 days, 44 goats and 3 seasons). Error bars represent the standard errors. Spce = Spinescent, Spl = Spineless, Fl = Fine leaves, Bl = Broad Leaves, Dec = Deciduous and Ev = Evergreen.

Table 3.3 The statistical variables of diet selection (proportion) of physical traits and/or phenology of plant species within seasons

Season	Species traits	Mean ± SE	*t*-test	*df*	*P*
Dry	Spinescent	0.110 ± 0.011	-4.30	79	<0.001
	Spineless	0.043 ± 0.007			
	Fine leaves	0.073 ± 0.007	0.23	111	= 0.821
	Broad leaves	0.076 ± 0.012			
	Deciduous	0.073 ± 0.023	-0.82	91	= 0.414
	Evergreen	0.086 ± 0.014			
Early wet	Spinescent	0.093 ± 0.008	-4.62	80	<0.001
	Spineless	0.043 ± 0.007			
	Fine leaves	0.097 ± 0.009	-3.41	82	= 0.001
	Broad leaves	0.055 ± 0.008			
	Deciduous	0.097 ± 0.009	3.41	82	= 0.001
	Evergreen	0.055 ± 0.007			
Late wet	Spinescent	0.136 ± 0.011	-7.39	91	<0.001
	Spineless	0.042 ± 0.006			
	Fine leaves	0.142 ± 0.013	-5.60	83	<0.001
	Broad leaves	0.058 ± 0.008			
	Deciduous	0.142 ± 0.013	4.93	88	<0.001
	Evergreen	0.066 ± 0.009			

SE = Standard error; df = Degree of freedom; P = Significance level.

Table 3.4 The proportion of relative abundance of browse species (using number of browseable plants) of two paddocks in Zululand Thornveld consumed in three seasons (one paddock was used twice, in the dry season and early wet season)

Species	Dry	Early wet	Late wet
A. natalitia	0.1937	0.1937	0.1692
A. nilotica	0.0303	0.0303	0.0438
C. odorata	0.2814	0.2814	0.2387
D. cinerea	0.0827	0.0827	0.0952
S. myrtina	0.0352	0.0352	0.0430
C. rudis	-	-	0.0176
L. camara	0.0079	0.0079	0.0039
Other browse[a]	0.0754 (14 sp)[b]	0.1352 (15 sp)	0.1418 (16 sp) ‡
P. guajava	0.0247	0.0247	-
P. lanceolata	0.0492	0.0492	-
R. pentheri	0.0056	0.0056	-
J. multipartitum	0.0185	0.0185	0.0292
Not-selected [c]	0.2140 (15 sp)	0.1356 (13 sp)	0.2132 (18 sp)

[a] Species selected by goat but occurring in small proportions (less than 15 times found in the field or the diet), including *Acokanthera rotundata, Apodytes dimidiata, Canthium inerme, Carissa bispinosa, Diospyros simii, Euclea daphnoides, Gymnosporia heterophylla, Gymnosporia maranguensis, Hippobromus pauciflorus, Lippia javanica, Phyllanthus reticulata, Rhus dentata, Rhus rehmanniana, Schinus terebinthifolius, Sideroxylon inerme* and *Ziziphus mucronata*.
[b] Includes *J. multipartitum* in the dry season and unknown species in the late wet season.
[c] Species not in the diet, including *Azima tetracantha, Cordia caffra, Cussonia spicata, Diospyros lycioides, Ehretia rigida, Grewia occidentalis, Gymnosporia harveyana, Gymnosporia glaucophylla, Phoenix reclinata, Putterlickia verrucosa, Rhoicissus tridentate, Schotia brachypetala, Scolopia zeyheri, Senna bicapsulata, Sesbania sesban,* and *Trichilia emetica*.

When the data were sorted and pooled according to browse species across the seasons, the relationships between the browse species selection index and their chemical traits were obtained (Table 3.7, Figure 3.2). Selection index for *A. nilotica* was positively correlated to ADF (r = 0.32, n = 44, P = 0.035), ADL (r = 0.32, n = 44, P = 0.035) and cellulose (r = 0.31, n = 44, P = 0.04). For *D. cinerea* NDF (r = 0.36, n = 44, P = 0.018), ADF (r = 0.29, n = 44, P = 0.059), ADL (r = 0.31, n = 44, P 0.042) and CP (r = 0.53, n = 44, P < 0.001) were positively related to selection index, while for *S. myrtina* the selection index was negatively related to NDF (r = -0.46, n = 43, P = 0.002), CP (r = -0.46, n = 43, P = 0.002) and CT (r = -0.47, n = 43, P = 0.002). Selection index for *C. odorata* was positively correlated to NDF (r = 0.46, n = 44, P = 0.002), ADF (r = 0.34, n = 44, P = 0.001) and cellulose (r = 0.43, n = 44, P = 0.004), while no significant relationship was noticed between the chemicals and selection index of *A. natalitia*, *Coddia rudis, J. multipartitum*,

L. camara, *P. guajava*, *P. lanceolata* and *R. pentheri*. However, there were no significant relationships between selection index and chemistry of plant leaves when the data were pooled according to the seasons, except CP in early wet season, which was negatively related to selection index (r = -0.22, n = 94, P = 0.030).

Table 3.5 The effect of season and plant species on selection index by goats (mean ± SE)

	Season		
Species	Dry	Early wet	Late wet
Acacia natalitia	0.483 ± 0.065	0.552 ± 0.058	0.633 ± 0.083
Acacia nilotica	2.173 ± 0.507	1.802 ± 0.290	3.094 ± 0.477
Chromolaena odorata	0.478 ± 0.083	0.191 ± 0.047	0.381 ± 0.047
Dichrostachys cinerea	0.888 ± 0.117	1.887 ± 0.253	2.345 ± 0.295
Scutia myrtina	6.783 ± 1.164	2.484 ± 0.605	2.880 ± 0.609
Lantana camara	2.935 ± 0.678	-	3.025 ± 0.982
Other browse	1.912 ± 0.475	1.632 ± 0.508	0.868 ± 0.188
Psidium guajava	0.251 ± 0.080	-	-
Pavetta lanceolata	0.632 ± 0.150	-	-
Rhus pentheri	4.055 ± 1.218	-	-
Jasminum	-	2.155 ± 0.345	1.477 ± 0.390
Coddia rudis	-	-	0.839 ± 0.231

Table 3.6 The effect of season, physical traits and phenology of plant species on selection index (mean ± SE)

	Season		
Trait	Dry	Early wet	Late wet
Fine leaves	1.962 ± 0.164	1.091 ± 0.246	1.459 ± 0.217
Broad leaves	1.367 ± 0.215	2.466 ± 0.340	1.778 ± 0.314
Deciduous	1.617 ± 0.151	1.091 ± 0.246	1.459 ± 0.217
Evergreen	1.556 ± 0.260	2.466 ± 0.340	0.986 ± 0.237
Spineless	1.024 ± 0.165	0.874 ± 0.200	1.778 ± 0.314
Spinescence	2.370 ± 0.238	2.278 ± 0.298	1.459 ± 0.217

Table 3.7 Chemical composition (g/kg DM) of selected browse species by goats in Zululand Coastal Thornveld

Species	Dry season					Early wet season					Late wet season				
	NDF	ADF	ADL	CP	CT	NDF	ADF	ADL	CP	CT	NDF	ADF	ADL	CP	CT
Acokanthera rotundata	434	349	214	59.9	1.7	-	-	-	-	-	-	-	-	-	-
Apodytes dimidiata	-	-	-	-	-	487	322	144	89.0	74.3	-	-	-	-	-
Acacia natalitia	432	294	213	122	118	412	281	197	141	212	534	431	339	133	97.0
Acacia nilotica	242	138	84	116	3.8	263	130	77	136	16.5	281	186	121	138	2.6
Carissa bispinosa	362	224	142	60.1	93.0	420	306	207	76.4	228	-	-	-	-	-
Canthium inerme	517	407	330	94.6	28.7	552	482	361	125	2.3	552	510	373	118	7.2
Chromolaena odorata	386	245	105	186	0.5	233	143	62	216	0.5	354	313	212	226	0.5
Coddia rudis	360	208	130	68.3	5.6	333	213	130	100	4.2	395	318	188	106	2.3
Dichrostachys cinerea	488	356	223	109	45.8	454	259	168	173	59.0	627	548	355	170	33.8
Diospyros simii	450	344	279	84.9	21.0	525	406	262	115	32.0	573	529	351	107	9.2
Euclea daphnoides	552	377	245	69.3	29.9	601	474	339	77.5	52.4	-	-	-	-	-
Gymnosporia	392	213	119	88.3	95.9	461	283	175	115	44.6	617	506	275	96.0	13.8
Gymnosporia	532	357	219	78.1	56.7	520	379	264	90.6	75.5	585	502	291	76.8	68.2
Hippobromus pauciflorus	444	284	150	101	138	444	292	151	121	169	474	467	219	112	93.6
Jasminum multipartitum	284	182	108	80.4	14.9	336	188	118	127	1.0	507	512	263	136	0.6
Lippia javanica	455	262	130	134	1.3	434	305	145	143	73.3	559	558	293	164	0.2
Lantana camara	342	218	107	116	14.5	362	251	84	150	5.2	429	386	201	156	2.9
Psidium guajava	462	305	167	78.8	24.7	483	332	184	121	106	540	546	327	123	43.5
Pavetta lanceolata	439	327	253	70.6	98.6	582	499	407	122	32.0	625	619	463	107	27.1
Phyllanthus reticulata	-	-	-	-	-	233	133	057	209	4.6	392	162	061	152	7.1
Rhus dentata	514	335	225	96.3	4.4	612	474	365	145	15.8	625	548	404	114	12.3
Rhus pentheri	421	285	213	94.5	104	574	415	287	157	1.8	636	572	399	152	0.5
Rhus rehmanniana	432	269	158	80.7	117	598	451	324	139	22.9	606	573	400	147	6.1
Sideroxylon inerme	412	297	184	96.8	72.0	-	-	-	-	-	-	-	-	-	-
Scutia myrtina	460	313	214	105	32.2	497	273	188	123	208	508	431	293	129	133
Schinus terebinthifolius	402	325	256	96.9	22.7	-	-	-	-	-	481	379	265	117	12.5
Ziziphus mucronata	517	198	111	96.6	26.0	440	192	86	127	23.1	572	446	186	123	15.5

NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin; CP - crude protein; CT - condensed tannin.

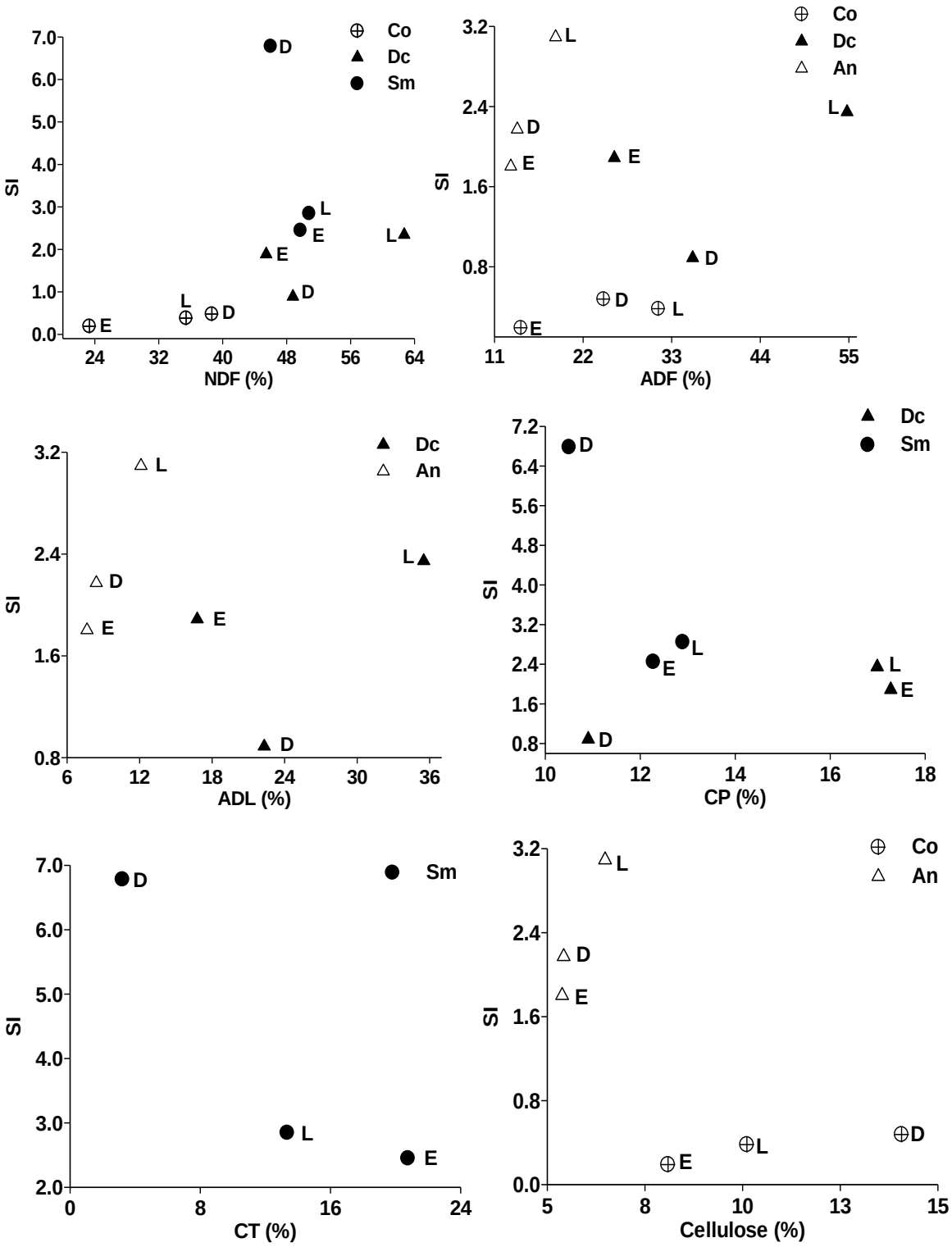

Figure 3.2 Relationships between chemical variables of browse species and selection index (SI) of goats in different seasons. An - Acacia nilotica; Co - Chromolaena odorata; Dc - Dichrostachys cinerea; Sm - Scutia myrtina; NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin; CP - crude protein; CT - condensed tannin; D - dry season; E – early wet season; L – late wet season.

3.4 Discussion

The goats foraged on 31 out of a total of 44 woody plant species in the study area. However, this number of foraged palnts varied among seasons. Other studies have also reported on seasonal variation in diet selection (Yayneshet *et al.*, 2008). Moreover, the goats had higher selectivity in the dry season than the wet season. Owen-Smith and Cooper (1988) showed that some plant species avoided during the wet season may be accepted in the dry season. This might be attributed to the low availability of food during the dry season (Abate, 1996). It was shown that *S. myrtina* comprised the highest proportion of the diet during the dry season whilst it comprised a smaller proportion of the diet in the early and late wet seasons. *Scutia myrtina* is an evergreen species and most of the other species are deciduous. Consequently, the choices of diet are narrow when there is low availability of palatable materials. Abate (1996) indicated a main factor that affects goat feeding behaviour during the dry season is low food availability. *Scutia myrtina,* an evergreen and broad leaf species, is more available in the dry season than other species and goats are likely to eat more of it. In the wet season the quality and availability of edible browseable material increases in general (Nyamangara & Ndlovu, 1995). Results from the current study agree partially with observations made in cafeteria style experiments (Dziba *et al.*, 2003a; Mkhize, 2008), when it was reported that *S. myrtina* was the most preferred species during the dry, early wet and late wet seasons.

In the early and late wet season, *D. cinerea* was the most selected species, while in the dry season its contribution to the diet was low. This observation might be due to variation in leaf availability, with fewer leaves in the dry season than in the wet season. These finding support previous observations (Yayneshet *et al.*, 2008; Basha *et al.*, 2009). Moreover, Mlambo *et al.* (2004) reported that *D. cinerea* comprised a large proportion of goat diets in Zimbabwe. In contrast, Ouédraogo-Koné *et al.* (2006) and Sanon *et al.* (2007) reported that *D. cinerea* was avoided by goats in West Africa savannah, but this could be due to variations in vegetation, climate and soil of the research sites. Owen-Smith and Cooper (1988) showed that some avoided plant species in the wet season may be accepted in the dry season. While it could be inferred that reduced availability in the dry season causes plants that are avoided in the wet season to become accepted in the dry season (Owen-Smith & Cooper, 1988), this was not clearly the case in our study. Many of the deciduous species in the study area are able to retain leaves for most of the dry season because of the

humid conditions of the environment. Clearly, factors affecting seasonal variations in diet are more complex than expected and require further research.

The proportion of *A. natalitia* in the diet varied among the seasons. The moderate proportion in the dry season might be due to *A. natalitia* having more leaves than other deciduous species at the time (pers. obs.), whilst the moderate proportion in the early wet season could be attributed to a function of flowering. In this study goats preferred *A. natalitia* less than other common species, which agrees with previous observations (Basha *et al.*, 2009). In contrast, these observations are in disagreement with observations from studies where choice was limited (Dziba, *et al.,* 2003a; Mkhize, 2008). During the late wet season *A. nilotica* had the greatest selection index, compared to the dry and early wet seasons. These variations between seasons could be ascribed to more leaves in the late wet season. The present findings agree with earlier observations (Basha *et al.*, 2009) but contrast with observations from controlled experiments where diet choice was limited (Jansen *et al.* 2007; Mkhize, 2008).

Chromolaena odorata is one of the most toxic weeds, because of alkaloids and/or compounds that affect feeding on this foliage (Prasad *et al.*, 2005). In our study, *C. odorata* had the highest biomass throughout all seasons but it was avoided in the wet season and accepted during the dry season. This avoidance could be attributed to chemical traits, but during the dry season the animals may be obligated to feed on *C. odorata* because of low feed availability (Abate, 1996). It is also possible that the chemical composition of *C. odorata* changes favourably during the winter, permitting herbivores to consume small amounts.

Physical characteristics of plant species are effective factors that influence diet selection. Wilson and Kerley (2003a) showed a significant relationship between foraging rate and plant morphology. Preference of spinescent species was significantly higher than that of spineless species within and across the seasons. This result agrees with Mkhize (2008) and Basha *et al.* (2009). Such morphological traits do not deter animals from foraging but reduce bite size and bite rate (Cooper & Owen-Smith, 1986). Furthermore, plant morphology correlates with diet selection (Shipley *et al.*, 1998; Dziba *et al.*, 2003a). In contrast to our results, Owen-Smith and Cooper (1987) reported that spines negatively influenced selection in the dry season and this could be ascribed to limited leaf availability.

Furthermore, spinescence restricts browsing (Woodward & Coppock, 1995), but kudu are more affected than goats and impalas because of differences in mouth size (Cooper & Owen-Smith, 1986).

In the current study goats consumed significantly larger proportions of species with fine leaves across the seasons compared to those with broad leaves. However, within the seasons, plant species with fine leaves comprised the larger proportion of the diet only in the wet seasons, but were more preferred in the dry season than species with broad leaves. The high proportion of fine-leaf species in the wet season diet could be ascribed to the increased availability of those species, while the higher preference in the dry season might be due to restricted choice of species. These observations in the dry season disagree with Mkhize (2008), while there is agreement in the wet seasons.

In general, deciduous species comprised a higher proportion of the diet across the seasons compared to evergreen species. Moreover, within seasons, deciduous species comprised the greater proportion of diet during the wet seasons. Shipley *et al.* (1998) reported that moose take the greatest proportion of bites and mass from deciduous species. In addition, animals preferred deciduous woody species more than evergreen species (Shipley *et al.*, 1998). However, evergreen species were more preferred during the early wet season compared to deciduous species, while other studies reported that preference was unrelated to phenology (Watson & Owen-Smith, 2002; Dziba *et al.*, 2003a; Mkhize, 2008). This preference might be due to variation in edible material availability, while deciduous species still had fewer leaves.

For chemical traits, the study hypothesized that preference of plant species depends upon the fibre concentration in plants which changes with seasons and thereby reducing forage quality through physical toughness and poor digestibility. Watson and Owen-Smith (2002) reported that fibres are the main chemical factors to influence the preference of woody species to eland. Moreover, fibre is an important factor that could influence *in vitro* fermentation (Ndlovu & Nherera, 1997). In this study no significant relationships were found between plant preference and fibre components (NDF, ADF, ADL, cellulose and hemicellulose) in all three seasons. These results agreed with Shipley *et al.* (1998). However, between plant species, fibre fractions fluctuated in their relation to feed preference. Preference of *A. nilotica*, *D. cinerea,* and *C. odorata* was positively related to

various fibre fractions. These findings support Alonso-Diaz *et al.* (2008) and Mkhize (2008). These plant species varied in their preference to goats; therefore, fibre minimizing strategies (Jansen *et al.*, 2007) could not explain preference. Moreover, fibre had no correlation to selection for moose (Shipley *et al.*, 1998) and goats (Mkhize, 2008), although Cooper *et al.* (1988) reported that food selection could be affected negatively by fibre components. On the other hand, *S. myrtina* preference among seasons was negatively related to fibre, which supports previous observations (Basha *et al.*, 2009).

Many studies have reported that selection of food depends upon protein levels (Nyamangara & Ndlovu, 1995; Dziba *et al.*, 2003a). Moreover, Kyriazakis *et al.* (1999) indicated that animals have developed behavioural mechanisms which allow them to recognize foods on the basis of their nutritional as well as other properties. In this study a negative relationship was observed between preference and protein in the early wet season. However, among plant species, *D. cinerea* and *S. myrtina* preference was positively and negatively related to protein, respectively. This clearly shows that there is no consistent relationship (i.e., relationships between seasonal variations in chemistry and preference are species-specific). Thus, no evidence was found to support the hypothesis of maximising nutrient intake, which agrees with some studies (Danell *et al.*, 1994; Shipley *et al.*, 1998; Jansen *et al.*, 2007), but not others (Dziba *et al.*, 2003a; Ganqa *et al.*, 2005). These differences among studies could be ascribed to variations in climate as well as different plant and animal species.

In this study, no relationship was observed between preference and condensed tannin in the three seasons. However, amongst plant species, seasonal changes in preference of *S. myrtina* were negatively related to condensed tannins suggesting that tannins only have negative effects on preference in some species. In addition, tannins are not avoided completely by goats but they are kept below a certain threshold (Jansen *et al.*, 2007). Also goats could limit the intake when plant species have high concentrations of tannin and lignin (Dziba *et al.*, 2003a). However, a varied diet is likely to minimise the effects of tannins. Moreover, an *in vitro* gas production study showed that addition of polyethylene glycol (PEG) to all species in our study emphasized the inhibitory effect of tannins on rumen microbes in tannin-rich feed (Chapter 4).

3.5 Conclusions

The contribution of each plant species to the diet of goats, and the preference of goats for each species, varies among seasons. Factors such as plant spinescence and leaf morphology seem to be having an effect on diet selection with changes within plant species and seasons. However, leaf phenology did not affect the preference. The effect of chemical variables (fibres, protein and condensed tannins) on preference varied among species, indicating that the reasons for preference are species-specific. Therefore, goats forage from a wide range of plant species and this behavior suggests goats can optimize utilization of the biodiversity of savannahs and can be useful for managing woody plants in natural pastures.

Chapter 4

Effects of season, browse species and tannins on gas production kinetics of forages in the sub-humid subtropical savannah, South Africa

Abstract

Seasonal, species and tannin effects on gas production (GP) and GP kinetics parameters of five plant species from sub-humid subtropical savannah of South Africa were investigated. The plant species were *Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata*, and their leaves were harvested during the dry (June/July), early wet (November/December) and late wet (February/March) seasons. Automated *in vitro* gas production technique was used in two experiments carried out with nine replicates (3 runs x 3 replicates). The first experiment was to test the effect of season and species, while the second experiment tested the biological effect of tannins using polyethylene glycol 4000 (PEG). The PEG treatment was applied to samples in the early and late wet seasons. There were wide variations between seasons and among species in crude protein (CP), natural detergent fibre (NDF), acid detergent fibre (ADF) and condensed tannins (CT). Seasons and species affected the maximum GP and GP kinetics parameters. *Chromolaena odorata* had the highest CP (185.8-226.4 g kg^{-1}), GP (87.3-104.1 ml g^{-1} DM), gas produce from soluble (47.6-50.9 ml g^{-1} DM) and insoluble (39.8-50.9 ml g^{-1} DM) fractions during the three seasons compared to other browse species. The maximum GP, its degradation rate (C) and gas from the soluble fraction were positively correlated to CP without and with PEG. Gas from the soluble fraction was negatively correlated to NDF, ADL and CT; and GP to CT without PEG. The addition of PEG (tannin binding agent) emphasizes that the inhibitory effect of tannins on rumen microbes was great for tannin-rich feed. The results recommend that these browse species have potential to be used as feed supplements.

Keywords: browse species, tannin-rich species, tannins, polyethylene glycol.

4.1 Introduction

In tropical and sub-tropical areas, profitable livestock productivity is mainly limited by forage quantity and quality. Generally, most ruminant livestock in tropical Africa survive on natural pastures (Aganga & Tshwenyane, 2003) but inadequate feed throughout the year is one of the major factors of low gain rate and low animal productivity that mainly occur in the dry season (Makkar & Becker, 1998). Legumes may provide valuable sources of protein to support ruminant production in tropical savannah (Ngwa *et al*., 2003). However, seasonal variations in availability and nutritive value of forages mainly limit livestock productivity (Winrock, 1992). Browse and shrub fodders are vital supplements for covering the seasonal shortage in ruminant feed availability and nutritive value (Topps, 1992). The nutrient composition of browse is normally estimated by chemical analysis. However, this does not give enough information about true nutritive value of feed. Beside, there is enough information on the seasonal effects on chemical composition of browse in sub-tropical savannah (Scogings *et al*., 2004; Mkhize, 2008; Basha *et al*., 2009), but, little work has been done to investigate the effect of season with biological based method such as *in vitro* gas technique. It is one of the biological methods currently under more consideration in evaluation of ruminant feeds (Getachew *et al*., 1998; Dijkstra *et al*., 2005).

In vitro gas production techniques have become accepted for evaluating forage digestion features and fermentation kinetics (Groot *et al*., 1996; Theodorou *et al*., 1998; Campos *et al*., 2004). It has been commonly applied for determining nutritive value of feeds (Sallam, 2005; Pashaei *et al*., 2010). More recently, the increased interest in alternative resources of ruminant feed has led to an increase in the use of gas production technique as a result of the benefit in assessing fermentation kinetics. Measuring gas gives useful information on fermentation kinetics of both fast and slow degradable fractions (Campos *et al*., 2000; 2004; Pashaei *et al*., 2010). The *in vitro* gas production analysis is one of the best technique among biological ones used in evaluating the nutritive value of tannin-rich feeds. Some *in vitro* studies indicate that some tannins are more active than others (Osborne & McNeill, 2001; Andrabi *et al*., 2005). High tannin concentration may have negative effects on the nutritive value of forages by changing their palatability, reducing intake and digestibility (Kumar & D'Mello, 1995). Tannin binding agents, such as polyethylene

glycol (PEG) have potential to determine phenolic-associated anti-nutritional effects in browse species by forming tannin-PEG complexes (Makkar *et al*., 1995a).

The objective of the study was to determine the effects of season, plant species and tannins on gas production and fermentation kinetics of browse species in sub-humid subtropical savannah. It was hypothesised that: (i) changes in quality of forage caused by seasonal variations in savannah may influence their rumen fermentation (Winrock, 1992; Larbi *et al*., 1998; Camacho *et al*., 2010); (ii) inherent differences in nutritional value among plant species could influence the result of rumen fermentation (Nsahlai *et al.*, 1994; Ndlovu & Nherera, 1997) which may relate to their chemicals characters (Nsahlai *et al.*, 1994; Ndlovu & Nherera, 1997; Apori *et al*., 1998; Larbi *et al*., 1998); and (iii) since tannin may exert negative effects on the nutritive value of forages by limitting intake and digestibility (Kumar & D'Mello, 1995), the addition of tannin-agent such as PEG might reduce these negative effects on rumen fermentation result (Makkar *et al.*, 1995a) of tannin-rich species.

4.2 Materials and methods

4.2.1 Plant species and chemicals analysis

Leaves of the main five plant species selected by goats in Zululand thornveld during the dry (June/July 2008), early (November/December 2008) and late wet (February/March 2009) seasons (Chapter 3) were sampled. The early and late wet seasons are each part of the wet (rainy) season. January is the middle of the wet season. The plant species were *Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata*. The browse samples were taken from three unbrowsed trees per species per season. Once collected, leaf samples were kept in paper bags and air dried prior to oven drying at 60˚C for 48 hours. Dried samples were ground through 1-mm mesh sieve (Retsch GmbH & Co. KG 5657 HANN 1, West-Germany) and stored in sealed plastic bottles until analyzed.

The milled samples were analyzed for chemical composition on the dry matter basis using pseudo replicates (3 replicates). Nitrogen (N) concentration was determined based on AOAC method 990.03 (AOAC, 1997) using a LECO, FP2000, nitrogen analyzer. Crude protein (CP) was calculated as 6.25 x N concentration. Neutral detergent fibre (NDF),

acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991) using ANKOM Technology Technique. The acid-butanol proanthocyanidin assay (Porter *et al.*, 1985) was used to determine condensed tannin (CT) (Makkar, 1995). Cellulose was calculated as the difference between ADF and ADL, while hemicellulose was derived from the difference between NDF and ADF.

4.2.2 Gas production

The incubation was done using automated gas production technique which was described by Pell and Schofield (1993). Two experiments were carried out; the first one tested the effect of season and species. The second experiment tested the effect of tannins by adding polyethylene glycol 4000 (PEG). Only a small amount of PEG (5.6 mg) was added to each bottle. High amount of PEG (500, 750, 1000 and 1250 mg/g) has been studied by Bueno *et al.* (2008) who found no difference among these levels of PEG. The PEG treatment was applied to samples of the early and the late wet seasons only because the dry season had limited samples due to some species being deciduous.

Milled samples (1.0 ± 0.001 g DM) were weighed into 250 ml Duran bottles for *in vitro* incubation. During a run, each sample, two controls (Lucerne (alfàlfà) and *Eragrostis* hay, Appendix 8), and blanks were represented by 3 Duran bottles. Three runs of incubations were applied to each season. A salivary buffer solution was prepared according to McDougall (1948). Salivary buffer was made by prepared solutions A and B. Solution A was made from 19.60, 7.40, 1.14, 0.94 and 0.26 g of NaHCO3, Na_2HPO_4, KCl and $MgCl.6H_2O$, respectively and dissolving in 2 L distilled water. Solution B was prepared by dissolving 2.65 g of $CaCl_2.2H_2O$ in 50 ml distilled water. Complete salivary buffer was made by adding 2 ml of solution B to solution A which was warmed up to 39°C with continuously stirring and saturated with carbon dioxide (CO_2), that immediately before starting. A 67 ml amount of the buffer was added to bottles bearing sample, control or blank. The bottles were kept in the incubator (39 °C) for 1 h to allow soaking of substrate prior to adding rumen fluid. Meanwhile a mixture of the rumen fluid was collected prior to morning feeding from three fistulated cows. The cows were allowed free access to graze on kikuyu pasture and supplemented with 2 kg of Lucerne hay per day, with *ad libitum* access to water and mineral lick. The rumen fluid was collected and filtered through four layers of cheesecloth into a pre-warmed flask (39 ºC) that had been flushed with CO_2. The

mixed rumen fluid was transported to the laboratory, and inoculation was completed by adding 33 ml of the rumen fluid to each bottle under a stream of CO_2 and then the bottle lid was tightened. The Duran bottles were incubated in the incubator channels and pressure sensors were fitted. Pressure logging was done at 20 min interval throughout an incubation period of 72 h. Calibration of the pressure logger had already been done as described by Pell and Schofield (1993) to establish gas pressure-volume relationship by regression equation. Total gas values were corrected for blank incubations which contained only buffer and rumen fluid. These corrected data were adjusted using the controls (Appendix 9). The adjusted data was fitted into the model of Schofield *et al*. (1994) to estimate the maximum GP and its degradation rate. These data were used to determine the GP kinetics by the model of Campos *et al*. (2000) using SAS (2002) as follows:

$$y = GP/ [1+e^{(2+4C (lt - t)}] \quad \text{(Schofield } et\ al.\text{, 1994) and}$$

$$y = A/ [1+\exp [2+4c_1 (lt - t)]] + B/ [1+\exp [2+4c_2(lt - t)]] \quad \text{(Campos } et\ al.\text{, 2000);}$$

where y is the gas volume (ml) at time *t*, GP is maximum gas production, C is constant degradation rate, A and B are the gas volumes (ml) from fast (soluble sugars and starch) and slowly (cellulose and hemicellulose) degradable fractions, respectively; c_1 and c_2 are the degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively; and lt is lag time (h) (for bacteria colonization).

The cumulative gas production (GP) was used to calculate the maximum rate of GP at the point of inflection (μ) and the time taken to produce half of gas volume ($T_{½}$) according to Sahoo *et al.* (2010) as follows:

$$\mu = GP \times C \text{ and}$$

$$T_{1/2} = lt + 1/(2*C)$$

4.2.3 Statistical analysis

The experimental design was a factorial design (5 species x 3 periods) for experiment 1 and (5 species x 2 periods x 2 PEG levels) for experiment 2. These data were subjected to the General Liner Model of SAS to detemine the effects of season, plant species and condensed tannins on gas production parameters, statistical significance being declared at

P<0.05. Means were compared by least significant difference (LSD). The models used were:

$$Y_{ijk} = \mu + s_i + p_j + (sp)_{ij} + \varepsilon_{ijk} \qquad \text{(Experiment 1);}$$

where, Y_{ijk} **is** the observation, μ is the population mean, s_i is the season effect (i = 1–3), p_j is the plant species effect (j = 1–5), $(sp)_{ij}$ is the interaction between season and plant pecies and ε_{ijk} is the residual error.

$$Y_{ijkl} = \mu + s_i + p_j + t_k + (sp)_{ij} + (st)_{ik} + (pt)_{jk} + (spt)_{ijk} + \varepsilon_{ijkl} \qquad \text{(Experiment 2);}$$

where, Y_{ijkl} is the observation, μ is the population mean, s_i is the season effect (i = 1–2), p_j is the plant species effect (j = 1–5), t is polyethelene glycol (PEG) effect (k = 1), $(sp)_{ij}$ is the interaction between season and plant pecies, $(st)_{ik}$ is the interaction between season and PEG, $(pt)_{jk}$ is the interaction between plant pecies and PEG, $(spt)_{ijk}$ is the interaction between season, plant pecies and PEG, and ε_{ijkl} is the residual error.

4.3 Results

4.3.1 Chemicals composition

The chemical composition of browse species is given in Table 4.1. In the late wet season, the plant species had the highest fibre fractions (NDF, ADF and ADL), while in the early wet season browse species attained the lowest values. However, with regards to NDF, *D. cinerea* had high value, while *A. nilotica* and *C. odorata* had low values during the dry season. The plant species had the highest CP value during the wet seasons, and the lowest during the dry season. *Chromolaena odorata* had the highest CP concentration and *S. myrtina* had the lowest concentration throughout the three seasons. The browse species in the early wet season had the highest CT level. *Acacia natalitia* had the highest CT in the early wet season, whilst *A. nilotica* had the lowest level during the three seasons. Cellulose was high during the late wet season, but low value in the early wet season, with one exception: *C. odorata* which had the highest during the dry season. Hemicellulose was high during the early wet season except *A. natalitia* and *C. odorata* had high values during the dry season, while low values of hemicellulose were recorded in the late wet season.

Table 4.1 Chemical compositions (g kg^{-1} DM) of five main browse species selected by goats sampled in three seasons at Zululand Thornveld

Parameters	Browse Species (Sp)	Season (Se) Dry	Early wet	Late wet	P<	RMSE	LSD	Sources of variation effects Se	Sp	Se x Sp
CP	Ana [a]	122	141	133	0.001	1.995	3.89	***	***	***
	An	116	136	138						
	Dc	109	173	170						
	Sm	105	123	129						
	Co [b]	186	216	226						
NDF	Ana	406	381	498	0.001	8.389	14.54	***	***	***
	An	228	243	272						
	Dc	452	427	586						
	Sm	429	452	471						
	Co	360	212	336						
ADF	Ana	276	260	401	0.001	32.172	9.23	***	***	*
	An	131	120	181						
	Dc	330	243	512						
	Sm	292	248	340						
	Co	219	130	298						
ADL	Ana	200	197	324	0.001	18.053	47.51	***	***	***
	An	79	77	119						
	Dc	207	161	337						
	Sm	200	181	278						
	Co	98	56	205						
CT	Ana	117.7	211.7	97.0	0.001	5.138	1.18	***	***	***
	An	3.8	16.5	2.6						
	Dc	45.8	59.0	33.8						
	Sm	32.2	207.6	133.4						
	Co	0.5	0.5	0.5						
Cellulose (Cell)	Ana	76	63	77	0.001	15.412	8.75	***	***	*
	An	51	43	61						
	Dc	123	82	175						
	Sm	92	66	122						
	Co	121	74	93						
Hemicellulose (Hcell)	Ana	130	121	96	0.003	32.117	35.22	***	ns	ns
	An	97	122	92						
	Dc	122	184	74						
	Sm	138	204	71						
	Co	141	83	39						

Ana - *Acacia natalitia*; An - *Acacia nilotica*; Dc - *Dichrostachys cinerea*; Sm - *Scutia myrtina*; Co - *Chromolaena odorata*; [a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; CP – crude protein; CT – condensed tannin; RMSE = root mean square error; LSD – least significant difference; ns ($P > 0.05$); * ($P < 0.05$); *** ($P<0.0001$).

4.3.2 Effect of season and plant species on gas production parameters (Experiment 1)

Table 4.2 shows the effects of season and plant species (experiment 1) on maximum GP, degradation rate (C), lag time (lt), maximum rate of GP at the point of inflection (μ) and half time ($T_{1/2}$). Season affected ($P<0.05$) the GP and strongly affected ($P<0.001$) the lt and the μ while browse species and its interaction with season affected ($P<0.001$) all variables (GP, C, lt, μ and $T_{1/2}$). Among the three seasons, GP was higher in the late wet season than in the early wet and the dry seasons. The lt was longer in the dry season than in the late wet and the early wet seasons. The maximum rate of GP (μ) was faster in the late wet season than in the early wet and the dry seasons.

Among the five plant species, *C. odorata* had the highest GP, while *A. nilotica, A. natalitia*, *D. cinerea* and *S. myrtina* had similar and low GP. *Chromolaena odorata* had the fastest degradation rate, followed by *A. natalitia*, *D. cinerea*, *A. nilotica* and *S. myrtina* in this order. *Acacia natalitia* had the longest lt whilst *C. odorata* had the shortest lt. *Chromolaena odorata* had the fastest μ, whilst the others had similar μ. *Chromolaena odorata* had shortest $T_{1/2}$ (13 h) while the others range from 26 to 34 h.

Interaction between season and browse species (Appendix 10) had different trends for the variables. For *A. natalitia* and *A. nilotica,* GP decreased from the dry to the early wet seasons then increased in the late wet season (*A. natalitia)* or remained similar between the early wet and the late wet seasons (*A. nilotica)*. *Dichrostachys cinerea* had similar GP in the dry and the early wet seasons from where it increased in the late wet season. For *S. myrtina,* GP decreased from the dry to the late wet seasons. The GP for *C. odorata* increased from the dry to the two wet seasons which were similar.

The degradation rate (C) of *A. natalitia* was fastest during the early wet season while it was lowest in the dry and the late wet seasons. For *A. nilotica*, *D. cinerea,* and *S. myrtina,* the degradation rates were low and similar, but for *C. odorata* the degradation rates were similar between the dry and the early wet seasons but highest during the late wet season. The maximum rate of GP (μ) of *A. natalitia* did not change across the three seasons. The μ of *A. nilotica* decreased from the dry to the early wet and late wet seasons, which were similar. For *D. cinerea*, both the dry and the early wet seasons had similar μ which were both lower than in the late wet season. For *S. myrtina,* μ was higher in the dry than in the

late wet seasons and for *C. odorata* μ increased from the dry through the early wet to the late wet season.

Table 4.2 The effects of season on *in vitro* gas production parameters of browse species sampled in three seasons at Zululand Thornveld and fermented using rumen fluid (Experiment 1)

		Season (Se)						Sources of variation effects		
Parameters	Species (Sp)	Dry	Early wet	Late wet	P <	RMSE	LSD	Se	Sp	Se x Sp
GP	Ana [a]	30	23	42	0.001	4.042	2.821	*	***	***
(ml g^{-1}DM)	An	50	41	43						
	Dc	26	29	49						
	Sm	44	- ¥	28						
	Co [b]	87	104	102						
C (h^{-1})	Ana	0.018	0.036	0.020	0.001	0.006	0.006	Ns	***	***
	An	0.021	0.015	0.017						
	Dc	0.019	0.017	0.022						
	Sm	0.019	-	0.014						
	Co	0.040	0.040	0.051						
lt (h)	Ana	9.1	3.2	5.7	0.001	0.507	1.022	***	***	***
	An	4.7	3.8	4.2						
	Dc	6.6	2.0	1.3						
	Sm	3.6	-	5.4						
	Co	0.7	1.7	1.7						
μ (ml h^{-1})	Ana	0.6	0.8	0.85	0.001	0.252	0.042	***	***	***
	An	1.0	0.6	0.72						
	Dc	0.5	0.5	1.1						
	Sm	0.8	-	0.4						
	Co	3.5	4.2	5.2						
$T_{1/2}$ (h)	Ana	37	24	31	0.001	2.814	2.204	Ns	***	***
	An	29	38	34						
	Dc	35	34	25						
	Sm	30	-	41						
	Co	14	14	12						

Ana - *Acacia natalitia*; An - *Acacia nilotica*; Dc - *Dichrostachys cinerea*; Sm - *Scutia myrtina*; Co - *Chromolaena odorata*; [a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; GP - maximum gas production; C - rate of gas production; lt - lag time; μ - maximum rate of gas production at the point of inflection of the gas curve; $T_{1/2}$ - half time to the maximum gas volume; ¥ - the curve of gas not fitted; RMSE - root mean square error; LSD – least significant difference; ns ($P > 0.05$); * ($P < 0.05$); *** ($P<0.0001$).

The lag time (lt) for *A. natalitia* decreased from the dry to the early wet seasons from where it increased. The lt were intermediate and similar for *A. nilotica* across the seasons. For *D. cinerea* the lag time decreased from the dry to the early wet and the late wet season, in this order. The lt of *S. myrtina* increased from the dry to the late wet seasons. The lt for *C. odorata* increased from the dry to the two wet seasons which were similar. The time taken to produce half maximum GP ($T_{1/2}$) for *A. natalitia* decreased from the dry to the early wet seasons before increasing in the late wet season. For *A. nilotica*, $T_{1/2}$ increased from the dry to the early wet seasons from where it decreased but slightly. *Dichrostachys cinerea* had similar $T_{1/2}$ in the dry and the early wet seasons from where it decreased in the late wet season. The $T_{1/2}$ for *C. odorata* was similar among the three seasons. For *S. myrtina* $T_{1/2}$ increased from the dry to the late wet seasons.

Within the five plant species and the three seasons, *C. odorata* had the greatest GP in the three seasons followed by *A. nilotica* in the dry season and *D. cinerea* in the late wet season. The lowest GP was observed in the early wet season with *A. natalitia* and in the dry season with *D. cinerea*. For degradation rate, *C. odorata* had highest values in the three seasons and *S. myrtina* had lowest value in the late wet season followed by *A. nilotica* in the early wet season. For the lag time (lt), *C. odorata* had rapid bacteria colonization in the dry season as advantage of nutritive value linked by shorter lt while *A. natalitia* had longest lt in the dry season. *Acacia nilotica* showed similar values of lt in the three seasons. The μ was high in *C. odorata* in the three seasons while *S. myrtina* had lowest value in the late wet season. *Scutia myrtina* had the longest $T_{1/2}$ in the late wet season, while *C. odorata* had shortest $T_{1/2}$ value in the three seasons as advantage of nutritive value indicating high intake.

4.3.3 Effects of PEG on gas production parameters (Experiment 2)

Table 4.3 shows the effect of PEG on maximum GP, degradation rate (C), lag time (lt), maximum rate of GP at the point of inflection (μ) and half time ($T_{1/2}$) (experiment 2). The addition of PEG affected ($P<0.001$) GP, C, μ and $T_{1/2}$ but did not affect the lag time. Season and its interaction with plant species (Se x Sp) affected ($P<0.001$) the C, μ and $T_{1/2}$, while plant species affected ($P<0.001$) all variables (GP, C, lt, μ and $T_{1/2}$) (experiment 2). The interaction between browse species and PEG (Sp x PEG) affected GP, C and μ in tannin-rich group (*A. natalitia*, *A. nilotica*, *D. cinerea* and *S. myrtina*) but exerted no

influence for *C. odorata*. The Se x Sp x PEG interaction affected (P<0.001) GP, C and $T_{1/2}$. The GP changes due to PEG followed this order: *S. myrtina* > *A. natalitia* > *A. nilotica* > *D. cinerea*. However, changes in the C were highest in *S. myrtina* followed by *D. cinerea*, *A. nilotica* and *A. natalitia*. Although μ attained similar values for tannin-rich feeds, the changes due to PEG were higher in the early wet than in the late wet seasons; μ did not change for *C. odorata*. The addition of PEG decreased the lt and $T_{1/2}$ of tannin-rich group but did not cause an appreciable change for *C. odorata.*

With the addition of PEG, *A. natalitia*, *A. nilotica* and *D. cinerea* showed higher increment in GP in the early wet season than in the late wet season; for *C. odorata* the changes were small. For tannin-rich feeds, the degradation rate (C) experience variable positive increment in the early and the late wet seasons except for *A. natalitia* in the early wet season which had a decrease (-13 %). *Scutia myrtina* had the highest increment in C during the late wet season. The lt and $T_{1/2}$ had similar trends; they had positive increments for *C. odorata* in both season but variably negative increments for tannin-rich group. *Acacia natalitia*, *A. nilotica* and *D. cinerea* had high increment in μ during the early wet season compared to the late wet season. *Scutia myrtina* had the highest increment in μ during the late wet season.

4.3.4 Effects of season and plant species on gas production kinetics (Experiment 1)

Table 4.4 presents the effect of season and plant species (experiment 1) on GP kinetics parameters. The parameters were the fermentation of the soluble (A) and insoluble (B) fractions, and their degradation rates c1 and c2, respectively. Season influenced (P=0.030) the A fraction and strongly influenced (P<0.001) the B fraction and its degradation rate (c2), whilst plant species and its interaction with season affected (P<0.001) all variables (A, B, c1 and c2). Among the seasons, the A fraction was higher in the early wet season than in the late wet and the dry seasons. The B was higher in the late wet season than in the dry and the early wet seasons, while c2 was faster in the dry season than in the early wet and the late wet seasons.

Table 4.3 The effects of polyethylene glycol (PEG) on *in vitro* gas production parameters of plant species sampled in two wet seasons at Zululand Thornveld (Experiment 2)

		GP (ml g^{-1} DM)			C (h^{-1})			lt (h)			μ			$T_{1/2}$ (h)		
Season	Species	-PEG	+PEG	Inc.	- PEG	+PEG	Inc.	- PEG	+PEG	Inc.	- PEG	+PEG	Inc.	- PEG	+PEG	Inc.
Early	Ana[a]	22	77	244	0.031	0.027	-13	2.9	1.6	-45	0.7	2.1	210	21	20	-5
wet	An	43	91	113	0.015	0.024	60	3.8	2.4	-37	0.6	2.2	248	38	23	-39
	Dc	34	72	111	0.016	0.028	75	2.1	0.6	-71	0.5	2.0	298	36	19	-48
	Sm	- ¥	90	-	-	0.027	-	-	2.2	-	-	2.4	-	-	21	-
	Co[b]	108	115	6	0.038	0.040	5	1.6	1.9	24	4.1	4.6	12	15	15	2
Late	Ana	47	67	44	0.020	0.031	55	4.3	1.2	-72	0.9	2.1	120	30	18	-40
wet	An	49	76	57	0.017	0.026	53	2.8	2.8	0	0.8	2.0	143	33	22	-34
	Dc	49	75	54	0.021	0.033	57	1.1	1.0	-4	1.1	2.5	137	25	16	-37
	Sm	29	88	201	0.012	0.027	125	4.0	3.5	-12	0.4	2.4	571	46	22	-52
	Co	98	120	22	0.051	0.046	-10	1.4	2.1	49	5.0	5.5	10	11	13	16
	P<	0.001			0.001			0.001			0.001			0.001		
	RMSE	8.976			0.004			0.935			0.463			3.079		
	LSD	2.538			0.001			0.264			0.131			0.871		
Sources of variation effects																
Se		ns			***			ns			***			***		
Sp		***			***			***			***			***		
PEG		***			***			ns			***			***		
Se x Sp		ns			***			ns			***			***		
Sp x PEG		***			***			***			***			***		
Se x Sp x PEG		***			***			ns			ns			***		

Ana - *Acacia natalitia*; An - *Acacia nilotica*; Dc - *Dichrostachys cinerea*; Sm - *Scutia myrtina*; Co - *Chromolaena odorata*; [a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; ¥ - the curve of gas not fitted; GP - maximum gas production; C - rate of gas production; lt - lag time; μ = maximum rate of gas production at the point of inflection of the gas curve; $T_{1/2}$ - half time to the maximum gas volume; Inc. - increment (%); RMSE – root mean square error; LSD – least significant difference; ns ($P > 0.05$); *** ($P < 0.0001$).

Table 4.4 *In vitro* gas production kinetics of browse species sampled in three seasons at Zululand Thornveld and fermented using rumen fluid (Experiment 1)

		Season (Se)						Sources of variation effects		
Parameters	Species (Sp)	Dry	Early wet	Late wet	P <	RMSE	LSD	Se	Sp	Se x Sp
A	Ana [a]	3	11	8	0.001	6.482	0.918	*	***	***
	An	26	35	8						
	Dc	6	5	17						
	Sm	10	-¥	2						
	Co [b]	48	53	51						
B	Ana	27	11	34	0.001	6.059	2.27	***	***	***
	An	24	6	36						
	Dc	21	24	33						
	Sm	34	-¥	27						
	Co	40	51	51						
c1	Ana	0.417	0.116	0.201	0.001	0.051	0.010	ns	***	***
	An	0.082	0.069	0.160						
	Dc	0.096	0.343	0.124						
	Sm	0.155	-	0.397						
	Co	0.115	0.117	0.133						
c2	Ana	0.019	0.025	0.020	0.001	0.004	0.002	***	***	***
	An	0.081	0.030	0.017						
	Dc	0.018	0.019	0.023						
	Sm	0.021	-	0.015						
	Co	0.032	0.033	0.042						

Ana - *Acacia natalitia*; An - *Acacia nilotica*; Dc - *Dichrostachys cinerea*; Sm - *Scutia myrtina*; Co - *Chromolaena odorata*; [a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; ¥ - the curve of gas not fitted; A and B - gas volume (ml) from fast (cell content) and slowly (cell wall) degradable fractions, respectively; c_1 and c_2 - degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively; RMSE - the root mean square error; LSD – least significant difference; ns ($P > 0.05$); * ($P < 0.05$); *** ($P<0.0001$).

Among the feeds, *C. odorata* had higher A fraction (51 g^{-1} DM), followed in order by *A. nilotica*, *S. myrtina*, *A. natalitia* and *D. cinerea* (25, 6, 7 and 9 ml g^{-1} DM, respectively). With respect to the B fraction, *C. odorata* (47 ml g^{-1} DM) produced the highest, followed in order by *A. nilotica*, *A. natalitia*, *D. cinerea*, and *S. myrtina*. The degradation rates were varied ($P<0.001$) among browse species; c1 ranged from 0.103 h^{-1} in *A. nilotica* to 0.276 h^{-1} in *S. myrtina;* and c2 also ranged from 0.018 h^{-1} in *S. myrtina* to 0.043 h^{-1} in *A. nilotica*.

The effect of interaction between season and plant species on A, B, c1 and c2 are presented in Table 4.4. The gas from the soluble fraction of *A. natalitia* and *A. nilotica* increased from the dry to the early wet seasons then decreased in the late wet season. *Dichrostachys cinerea* had similar GP from the soluble fraction between the dry and the early wet

seasons, but the GP from soluble fraction increased in the late wet season. The gas from the soluble fraction of *S. myrtina* decreased from the dry to the late wet seasons. No change occurred in gas from A fraction for *C. odorata*. The GP from the fibre fraction of *A. natalitia* and *A. nilotica* decreased from the dry to the early wet seasons and then increased to the late wet season. For *D. cinerea* GP increased consistently from the dry through the early wet to the late wet seasons. For *S. myrtina* GP from the fibre fraction decreased from the dry to the late wet seasons. The GP from the fibre fraction of *C. odorata* increased from the dry to the early wet and late seasons, both of which were similar.

For degradation rate of the soluble fraction (c1), *A. natalitia, D. cinerea*, and *S. myrtina* had the fastest rate in the dry, early wet and late wet seasons, respectively. During the dry season, c1 followed this order: *A. nilotica* < *D. cinerea* < *S. myrtina;* during the early wet season, the c1 followed the order: *A. nilotica* < *A. natalitia* and in the late wet season the order was*: A. natalitia*> *A. nilotica* >*D. cinerea.* The c1 did not change across the three seasons for *C. odorata*. The degradation rate of the fibre fraction (c2) for *A. natalitia* increased from the dry to the early wet seasons and then decreased in the late wet season. The c2 of *A. nilotica* decreased from the dry through the early wet to the late wet seasons. For *D. cinerea* and *C. odorata* the c2 were similar between the dry and the early wet seasons but increased during the late wet season. The *S. myrtina,* the c2 decreased from the dry to the late wet season.

4.3.5 Effects of PEG on gas production kinetics (Experiment 2)

The effect of PEG (experiment 2) on GP kinetics is given in Table 4.5. The results indicate that PEG increased GP from the soluble and fibre fractions of the tannin-rich group ($P<0.001$) in the two seasons except for *A. nilotica* where GP from the soluble fraction decreased by 34% in the early wet season. In the presence of PEG (Table 4.5), season affected ($P<0.001$) only the degradation rate of fibre (c2), while plant species influenced ($P<0.001$) all variables (A, B, c1 and c2). The interaction between season and plant species affected ($P<0.001$) only the degradation rates. The addition of PEG increased the GP from the soluble and fibre fractions but caused variable changes to the rates of GP. The effect of interactions between season, species and PEG ($p<0.001$) on the GP parameters (B, c1 and c2) is described below.

Table 4.5 The effect of polyethylene glycol (PEG) on *in vitro* gas production kinetics of five browse species (Experiment 2)

Season (Se)	Species (Sp)	A			B			c1			c2		
		- PEG	+ PEG	Inc.	- PEG	+ PEG	Inc.	- PEG	+ PEG	Inc.	- PEG	+ PEG	Inc.
Early wet	Ana[a]	9.6	32.2	235	12.8	44.9	251	0.140	0.110	-21	0.027	0.026	-4
	An	31.1	20.4	-34	11.5	70.3	511	0.069	0.121	75	0.067	0.024	-64
	Dc	9.8	30.8	214	24.1	40.8	69	0.323	0.106	-7	0.018	0.027	50
	Sm	¥	29.0	-	¥	61.4	-	-	0.073	-	-	0.025	-
	Co[b]	54.8	57.3	5	53.3	57.5	8	0.112	0.107	-5	0.032	0.032	0
Late wet	Ana	11.5	30.9	169	35.2	36.1	3	0.123	0.118	-4	0.020	0.028	40
	An	13.7	18.6	36	34.8	57.8	66	0.119	0.123	3	0.018	0.027	50
	Dc	16.2	33.0	104	32.3	41.6	29	0.134	0.131	-2	0.023	0.031	35
	Sm	2.4	20.7	763	26.7	66.8	150	0.219	0.077	-65	0.013	0.026	100
	Co	51.2	58.4	14	46.8	61.2	31	0.134	0.110	-18	0.041	0.037	-10
	P <	0.001			0.001			0.001			0.001		
	RMSE	7.676			8.824			0.039			0.003		
	LSD	2.170			2.495			0.011			0.001		
Sources of variation effects													
Se		ns			ns			ns			***		
Sp		***			***			***			***		
PEG		***			***			***			ns		
Se x Sp		ns			ns			***			***		
Sp x PEG		***			***			***			***		
Se x Sp x PEG		ns			***			***			***		

Ana - *Acacia natalitia*; An - *Acacia nilotica*; Dc - *Dichrostachys cinerea*; Sm - *Scutia myrtina*; Co - *Chromolaena odorata*; [a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; ¥ - the curve of gas not fitted; A and B - gas volume (ml) from fast (cell content) and slowly (cell wall) degradable fractions, respectively; c_1 and c_2 - degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively; Inc. - increment (%); RMSE - root mean square error; LSD - least significant difference; ns ($P > 0.05$); *** ($P < 0.0001$).

Gas production from the soluble fraction increased from 5% (*C. odorata*) to 235% (*A. natalitia*) during the early wet season; and from 14% (*C. odorata*) to 763% (*S. myrtina*) during the late wet season. The GP from the fibre fraction increased from 8% (*C. odorata*) to 511% (*A. nilotica*) during the early wet season and from 3% (*A. natalitia*) to 150% (*S. myrtina*) during the late wet season. With the exception of *A. nilotica* where the rate of GP from the soluble fraction were positive (75 and 3% during the early and late wet seasons, respectively), the changes in the rate were negative in the early (-5% to -21%) and the late wet season (-2% to -65%). Adding PEG decreased the rate of GP from fibre in *A. nilotica* (by 64%), has limited effect for *A. natalitia* and *C. odorata,* but increased the rate for *D. cinerea* (by 50%) during the early wet season. During the late wet season, PEG increased the rate of GP from fibre of tannin-rich feeds (35 to 100%) but decreased the rate for *C. odorata* slightly (-10%).

4.3.6 Correlations between chemical composition and GP kinetics

Table 4.6 shows the correlations between the CP, NDF, ADF, ADL, CT, cellulose (Cell) and hemicellulose (Hcell), and GP kinetics without and with PEG. Significant positive correlations occurred between GP, A, C, and μ, and CP without and with PEG. Without PEG, negative correlation occurred between GP and CT; lt and $T_{1/2}$, and CP; A and NDF, ADL and CT; c2 and NDF. With PEG, the negative correlation occurred only between $T_{1/2}$ and CP.

Table 4.6 The correlation coefficients between gas parameters, and chemical composition of five browse species at sub-humid sub-tropical savannah

		Chemical composition						
Treatment	Parameters	CP	NDF	ADF	ADL	CT	Cell	Hcell
- PEG	GP	0.82***	-0.42 ^{ns}	-0.26 ^{ns}	-0.36 ^{ns}	-0.63*	0.04 ^{ns}	-0.47 ^{ns}
	A	0.82***	-0.58*	-0.46 ^{ns}	-0.56*	-0.59*	-0.11 ^{ns}	-0.33 ^{ns}
	B	0.50 ^{ns}	-0.03 ^{ns}	0.12 ^{ns}	0.06 ^{ns}	-0.43 ^{ns}	0.24 ^{ns}	-0.48 ^{ns}
	C	0.74**	-0.26 ^{ns}	-0.14 ^{ns}	-0.21 ^{ns}	-0.16 ^{ns}	0.07 ^{ns}	-0.39 ^{ns}
	c_1	-0.30 ^{ns}	0.37 ^{ns}	0.30 ^{ns}	0.36 ^{ns}	0.47 ^{ns}	0.07 ^{ns}	0.20 ^{ns}
	c_2	0.08 ^{ns}	-0.55*	-0.46 ^{ns}	-0.46 ^{ns}	-0.40 ^{ns}	-0.35 ^{ns}	-0.23 ^{ns}
	lt	-0.58*	0.12 ^{ns}	0.09 ^{ns}	0.22 ^{ns}	0.40 ^{ns}	-0.27 ^{ns}	0.10 ^{ns}
	μ	0.85***	-0.36 ^{ns}	-0.20 ^{ns}	-0.29 ^{ns}	-0.47 ^{ns}	0.07 ^{ns}	-0.47 ^{ns}
	$T_{1/2}$	-0.69***	0.22 ^{ns}	0.11 ^{ns}	0.21 ^{ns}	0.33 ^{ns}	-0.16 ^{ns}	0.33 ^{ns}
+ PEG	GP	0.77**	-0.54 ^{ns}	-0.42 ^{ns}	-0.47 ^{ns}	-0.31 ^{ns}	-0.23 ^{ns}	-0.25 ^{ns}
	A	0.80**	-0.37^{ns}	-0.22 ^{ns}	-0.27 ^{ns}	-0.34 ^{ns}	-0.07 ^{ns}	-0.33 ^{ns}
	B	0.36 ^{ns}	-0.48 ^{ns}	-0.45 ^{ns}	-0.48 ^{ns}	-0.12 ^{ns}	-0.31 ^{ns}	-0.02 ^{ns}
	C	0.79**	-0.27 ^{ns}	-0.05 ^{ns}	0.08 ^{ns}	-0.33 ^{ns}	0.04 ^{ns}	-0.51 ^{ns}
	c_1	-0.01 ^{ns}	0.06 ^{ns}	0.06 ^{ns}	0.03 ^{ns}	-0.39 ^{ns}	0.11 ^{ns}	0.01 ^{ns}
	c_2	0.85**	-0.37 ^{ns}	-0.15 ^{ns}	-0.18 ^{ns}	-0.48 ^{ns}	-0.03 ^{ns}	-0.52 ^{ns}
	lt	-0.10 ^{ns}	-0.22 ^{ns}	-0.13 ^{ns}	-0.07 ^{ns}	0.19 ^{ns}	-0.26 ^{ns}	-0.19 ^{ns}
	μ	0.85**	-0.42 ^{ns}	-0.23 ^{ns}	-0.27 ^{ns}	-0.39 ^{ns}	-0.07 ^{ns}	-0.44 ^{ns}
	$T_{1/2}$	-0.69*	0.19 ^{ns}	0.03 ^{ns}	0.06 ^{ns}	0.23 ^{ns}	-0.05 ^{ns}	0.35 ^{ns}

GP - the maximum gas volume (ml g^{-1} DM); A and B - gas volume (ml) from fast and slowly degradable fractions, respectively; C, c_1 and c_2 - degradation rates (h^{-1}) for overall, fast and slowly degradable fractions, respectively; lt - lag time (h); $t_{1/2}$ (h) – half time of half maximum gas volume; μ - maximum rate of gas production at the point of inflection of the gas curve; NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin; CP - crude protein; CT - condensed tannin; Cell - cellulose; Hcell - hemicellulose; level of significance: ^{ns}, P>0.05; *, P<0.05; **, P<0.001; ***, P<0.0001.

4.4 Discussion

The chemical properties such as CP, fibre factions and tannins of browse species overlapped reported ranges for other browse species (Larbi *et al.*, 1998; Abdulrazak *et al.*, 2000b; Osuga *et al.*, 2006; Sallam *et al.*, 2010). The use of browse species by ruminants as nitrogen source is the main function of browses in numerous areas of the tropics, particularly during the dry seasons when other sources of nitrogen are not accessible because of availability or high prices. The CP content ranged from 105 to 226.4 g kg^{-1} DM, which is greater than the minimum required level for best microbial activity in the rumen (80 g kg^{-1} DM) (Annison & Bryden, 1998). When the CP content is below this range, the browse species may be utilized to complement poor quality pastures and crop residues (Ammar *et al.*, 2004a).

The browse species had different fibre contents during various seasons. These species variation in fibre concentrations among seasons may open up opportunity for the animals to forage more from one plant than another. Similar to the findings in the current study Vitti *et al.* (2005) observed that the fibre concentration of multipurpose trees and shrubs was higher in the wet than in the dry season. These variations are a useful offer of the browse species since Bakshi and Wadhwa (2004) reported that the voluntary intake and digestibility are controlled by fibre concentration particularly NDF and ADL. Additionally, the browse forage fibres have been shown to be more digestible (El Hassan *et al.*, 2000) than that of grasses and crop residues.

The plant species had a wide variation of CT, from 0.5 to 207.6 g kg^{-1} DM. The CT represents the main restriction to browse utilization in ruminant feeds as protein supplements. This occurs because in the rumen, tannins may form undegradable complexes with the feed nutrients especially protein, enzymes and metabolic products (Mangan, 1988). This might reduce the ability of rumen micro-organisms by inhibiting microbial activity (Griffiths, 1986; Sallam *et al.*, 2010). However, a small quantity (30-40 g kg^{-1} DM) of tannins has nutritional benefits by protecting forage proteins from extreme ruminal degradation without affecting forage intake or fibre digestion (Barry *et al.*, 1986; Frutos *et al.*, 2004).

Savannah seasonality is known to significantly influence the nutritive value whereby temperature and rainfall are associated with changes in quality (nutritive value) and quantity. These changes in the nutritive values of browse species could be linked to numerous factors such as species, soil, maturity stage (Singh *et al.*, 2005) and seasonality (Larbi *et al.*, 1998; Ammar *et al.*, 2004a; Yayneshet *et al.*, 2009). These factors may cause the changes in the quality through the differences in chemical variables by fluctuations in climatic circumstances between seasons, and their influences on cell wall lignification (Larbi *et al.*, 1996). Furthermore, the nutritional value of different tropical plants have been evaluated using the *in vitro* gas production technique (Nsahlai *et al.*, 1994; Larbi *et al.*, 1998; Abdulrazak *et al.*, 2000b; Vitti *et al.*, 2005; Osuga *et al.*, 2006). The variety of GP characteristics between seasons reported in this work are consistent with previous reports on tropical browse species (Larbi *et al.*, 1998; Arigbede *et al.*, 2006) except in *C. odorata* (non-browse species) the GP characteristics were out of the range. The variation in GP characteristics were possibly partly caused by differences in CP, NDF, ADF and CT between seasons (Table 4.1). For instance, *C. odorata* had high GP throughout the wet season and low value in the dry season meanwhile *C. odorata* had high CP concentration in the wet season and low in the dry season. In addition, the GP for *A. natalitia* and *S. myrtina* associated negatively with CT level. These results were confirming the positive and negative correlation between GP, and CP and CT, respectively (Table 4.6).

The high fermentation parameters associated with the late wet season might be associated with the high crude protein, fibre and low CT. Maghsoud and Akbar (2008) observed that the chemical composition (CP and fibre) significantly influences degradation parameters. The *in vitro* GP measured after 72 h across the seasons and plant species positively correlated to the CP. Ammar *et al.* (2004a), Gasmi-Boubaker *et al.* (2005) and Maghsoud and Akbar (2008) studied GP for 24 h in Mediterranean browse species and by-products; they observed positive relationship between GP and CP. Conversely, negative relationships between CP level and GP (Getachew *et al.*, 2004) and also no effect of CP on GP (Ndlovu & Nherera, 1997; Larbi *et al.*, 1998; Blümmel *et al.*, 1999) have been reported.

Species with low GP were associated with high level of CT and vice versa. The negative relationship between CT and GP has been reported elsewhere (Ngwa *et al.*, 2003; Njidda, 2010). Ngwa *et al.* (2003) recommended that tannins may limit the growth and/or activity of ruminal microorganisms. El Hassan *et al.* (1995) reported that phenolics reduced growth

of rumen cellulolytic species of *Ruminococcus* and influence GP from multipurpose fodder trees. Less gas produced from the soluble fraction has been reported for *Lespedeza* diet (Ouda & Nsahlai, 2007) meanwhile *Lespedeza* has high tannin content (Turner *et al.,* 2005). This is confirmed by *C. odorata* which has high GP and lower CT (0.5 g kg^{-1}). These results are consistent with gas produced from fibre fractions (Apori *et al.,* 2000) for an 8-week re-growth leaf of *C. odorata*. Previous studies suggest that tannins may bind and form undegradable complexes with protein (Makkar *et al.*, 1995a, b; Ngwa *et al.*, 2003; Bueno *et al.*, 2008), thereby reducing the potential GP.

The GP from the soluble fraction was limited by the fibre fractions (NDF, ADL) which could indicate a decrease in the activity of microbes by rising the hard conditions of the rumen environment while digestion progresses (Sallam *et al.*, 2010). This agrees with Ndlovu and Nherera (1997) who reported that 25-34% of the variation in GP in Zimbabwean browse species might be due to variation in quantity and quality of cell wall contents. For instance, *A. natalitia* in the early wet season and *D. cinerea* in the dry season had the lowest maximum GP meanwhile they have high cell wall content (NDF, ADF and ADL) in these seasons. This is consistent with Larbi *et al.* (1998), Haddi *et al.* (2003), De Biover *et al.* (2005), Njidda (2010) and Sallam *et al.* (2010) who indicated that GP was negatively correlated with NDF content. Furthermore, the high ADL concentration limited the gas from soluble degradable fraction, while Fadare and Babayemi (2007) reported that GP from slowly degradable fraction decreased with more ADL above 20%.

The rate of GP ranged from 0.014 to 0.051 h^{-1} which overlaps with the range (0.021 - 0.15 h^{-1}) of GP rate of Kenya browse species (Osuga *et al.*, 2006). Maghsoud and Akbar (2008) suggesting that chemical composition may affect the degradation rate of substrate. López *et al.* (1998) reported that the degradation rate of forages is affected by the plant cell wall fractions. With the exception of CP that was strongly related to the rate of GP (r = 0.74), all the other chemicals had non significant relationships with the rate of GP. These results agreed with Ammar *et al.* (2004b) who studied the relationship between chemical composition and *in vitro* digestibility of Spanish browse species. Larbi *et al.* (1998) found positive relationship between the rate of GP and CP of multipurpose trees and shrubs in the humid tropics of Nigeria. Conversely, others (Larbi *et al.,* 1998; Haddi *et al.,* 2003; Sallam *et al.,* 2010) reported negative correlation between degradation rate and fibre fractions

(NDF and ADF), whilst Nsahlai *et al.* (1994) and Sallam *et al.* (2010) observed that the degradation rate may limited by CT.

The estimates of kinetic parameters (lt, μ and $T_{1/2}$) of browse species have increased the information on the fermentative activity. These parameters differed among plant species and seasons, and may be due to differences in soluble matter and fibre concentration in browse species. The lt and $T_{1/2}$ were negatively related to CP (r=-0.58 to -0.69), while μ was positively related to CP (r=0.85). These suggest that the kinetic parameters are partly determined by the CP of the forage. However, the rumen could be filled with cell-wall polysaccharides that might influence the rumen microbial attachment and colonization of digesta particles (Cheng *et al.*, 1984). Haddi *et al.* (2009) found no differences in the maximum rate of *in vitro* degradation of the arid browses throughout the year.

The fermentation of tannin-rich species treated with PEG (experiment 2) resulted in increased GP of 44 – 244%. These results agreed with Makkar *et al.* (1995a) and Tolera *et al.* (1997) who reported that incubating treated tannin-rich plant material with PEG *in vitro* resulted in increases in GP up to 100%. Furthermore, the reaction of PEG treatment increased with augmenting CT content in browse species. Tolera *et al.* (1997) reported that the CT concentration depressed *in vitro* gas production rather than the total phenolics and total tannins as found in *Chamaecytisus palmensis*.

The present results show different effects of the interaction between season and browse species on all GP kinetics variables (experiment 1, Table 4.4 and 4.5) that may be due to differences of chemical composition around the year particularly CP, fibre fractions and anti-nutritional components. These differences in chemical composition across seasons were reported by others (Larbi *et al.*, 1998; Yayneshet *et al.*, 2009). Based on results of GP characteristics in the three seasons and among species, *C. odorata* was superior in quality compared to other browse species. However, *C. odorata* is not acceptable to goats because of problems associated with the presence of alkaloids (Prasad *et al.*, 2005). Further studies on *C. odorata* would be necessary to find methods of improving acceptability to livestock.

As shown with tannin-rich species, the early wet season showed a high increment in GP with adding PEG than the late wet season. This variation may be due to high CT in the early wet than the late wet seasons. These findings show the affinity of PEG with tannins

which may lead to increase the extent of accessible nutrients to microorganisms, mainly the accessible nitrogen. The findings disagreed with Getachew *et al.* (2001) and Nahand *et al.* (2010) who reported that PEG treatment had no influence on GP from both soluble and insoluble fractions for browse species. Jones *et al.* (2000) suggested that the evaluation of the activity of tannins and their effects on the digestion process could be estimated as the extent of GP with addition of PEG. In the literature, there were different responses to PEG treatment of foliage of acacia species to fermentation issue (Kamalak *et al.*, 2005; Rubanza *et al.*, 2005; Vitti *et al.*, 2005). These differences in the extent of fermentation of acacia species are possibly due to differences in tannin type and concentrations (Dalzell & Kerven, 1998). Based on Reed *et al.* (1990) it can be inferred that considerable amounts of tannins in processed browse would be in the NDF and ADF fractions, where they are bound to the cell wall and cell protein, and appear to be involved in decreasing digestibility. However, *A. nilotica* in the PEG treatment group in the early wet season showed a decrease in gas from soluble fraction that may partly be attributed to other soluble factors not measured in this study that may have the ability to attach to PEG and form undegradable complexes. The fact that the tannin-poor feed (*C. odorata*) is not affected by the addition of PEG could be attributed to the lack of inhibitory compound. These results are in agreement with the findings of Sallam *et al.* (2010) who found no effect of PEG on GP in alfalfa hay and *Atriplex caseneace*. Alfalfa is an herb and *A. caseneace* is a shrub and both have or supposed to have lesser CT content than browse.

The lt and $T_{1/2}$ decreased with PEG treatment for tannin-rich species only. It can be shown that short lt and $T_{1/2}$ matched with high gas production and maximum rate (μ) and may predict high nutritive value and microbial efficiency. This is supported by the findings of Ouda and Nsahlai (2009) and Cone and Van Gelder (2000), which showed high microbial efficiency in substrates with higher fermentation rates or with shorter $T_{1/2}$.

4.5 Conclusions

A significant variation in *in vitro* GP and fermentation kinetics were reported among seasons and among different browse species harvested from sub-humid subtropical savannah of KwaZulu-Natal Province, South Africa. These variations were related with the CP content and/or tannin content. The levels of condensed tannin concentration appeared to be the major factors limiting the *in vitro* GP of tannin-rich browse species. The addition

of PEG improved the extent of fermentation, especially of tannin-rich browses. It is suggested PEG should be used in goats foraging pastures. Also, *C. odorata* can be used as supplementary protein source like high-quality leguminous forages. The *in vitro* GP results showed that *C. odorata* leaves may have little or no anti-fermentation compounds; however the reluctance of livestock to consume *C. odorata* (personal observation) is not related to its high protein and GP levels and must be for other reasons. Further studies would need to investigate these reasons and establish new methods for promoting intake rate of *C. odorata*.

Chapter 5

Effects of season, browse species and polyethylene glycol (PEG) on *in vitro* degradability of forages in the sub-humid subtropical savannah, South Africa

Abstract

The effects of season, browse species and polyethylene glycol (PEG) on *in vitro* digestibility of five plant species from sub-humid subtropical savannah of South Africa were investigated. The leaves of plant species (*Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata*) were harvested during the dry (June/July), early wet (November/December) and late wet (February/March) seasons. An *in vitro* technique was used in two experiments carried out with nine replicates (3 runs x 3 replicates). Experiment one tested the effect of season and browse species and the second experiment tested the effect of tannins using polyethylene glycol 4000 (PEG) on *in vitro* degradability of the forages. The PEG treatment was applied to samples of the early and late wet season only. Season and species affected the true degradability (TrDeg), microbial yield (MY), portioning factor (PF) and short chain fatty acids (SCFA). The TrDeg ranged from 634 to 856 g kg^{-1} DM. The total SCFA varied between seasons and among browse species. The addition of PEG decreased the TrDeg and PF but increased total SCFA. Addition of PEG (tannin binding agent) reduced the efficiency of microbial protein synthesis in tannin-rich feeds. The results suggest that these browse species have potential to be used as feed supplements.

Key words: tannin-rich species, tannins, microbial yield, short chain fatty acids.

5.1 Introduction

The production of feed in tropical areas is associated with irregular and variable rainfall that restricts seasonal biomass yield of herbaceous and browse species. Sometime livestock in these areas must survive on limited feed of poor quality for long periods of the year

(Nyamukanza *et al.*, 2008). However, browse trees are essential in animal feeding programs because they are better supplementary feeds than most pasture vegetations and maintain their nutrients into the dry season when feed resources are exhausted (FAO, 1997). In tropical Africa, most ruminant livestock survive on natural pastures (Aganga & Tshwenyane, 2003) but insufficient feed during the year is one of the major factors of low gain rate and low animal productivity that mainly occur in the dry season (Makkar & Becker, 1998). Furthermore, insufficient rainfall during the dry period in semi-arid regions of South Africa provides a poor supply of good quality feed which leads to poor livestock productivity. Legumes may supply important protein sources to support ruminant production in tropical savannahs (Ngwa *et al.*, 2003). However, variations in quantity and nutritional value of forages between seasons are main reasons for limited livestock productivity (Winrock, 1992). Browse and shrub fodders are important feed supplies for covering the seasonal shortage in ruminant feed availability and nutritive value (Topps, 1992; Mlambo *et al.*, 2008). The evaluation of nutritive value of browse is usually estimated by chemical analysis, though this may not provide sufficient information about true nutritive value of feed. On the other hand, there is sufficient information on the seasonal effect on chemical composition of browse in sub-tropical savannah (Scogings *et al.*, 2004; Mkhize, 2008; Basha *et al.*, 2009; 2012). Little work has been done at sub-humid subtropical savannah to investigate the effect of season on degradability with biological based method such as *in vitro* gas technique.

In vitro gas technique is receiving more attention in estimating quality of ruminant feeds (Getachew *et al.*, 1998; Dijkstra *et al.*, 2005). The technique has become established for evaluating forage digestion features (Groot *et al.*, 1996; Theodorou *et al.*, 1998). It has been commonly applied for determining nutritive value of feeds (Sallam, 2005; Pashaei *et al.*, 2010). The *in vitro* gas technique is the best technique to use for estimating degradability and is easier to measure the short chain fatty acids (SCFA) yield. Moreover, it is useful to use the result from gas production technique such as degradability and gas production to calculate partitioning factor as useful rank tool for feedstuffs (Dijkstra *et al.*, 2005). Additionally, the calculation of degradation efficiency factor is useful to show the rapidity and the amount of feed degradation in order to make nutrients available (Ouda & Nsahlai, 2009). However, *in vitro* studies indicated that tannin effects depend upon types and concentrations (Osborne & McNeill, 2001; Andrabi *et al.*, 2005). High tannin concentration may exert negative effects on the nutritive value of forages by changing their

palatability, or reducing intake and digestibility (Kumar & D'Mello, 1995). Polyethylene glycol (PEG) has been applied to inhibit the negative effects of tannins on feed intake and digestibility (Makkar *et al.*, 1995a; Getachew *et al.*, 2000a; Baba *et al.*, 2002), thereby providing an indirect method of testing the effect of tannins. The objective of the study was to determine the effect of season, plant species and PEG on *in vitro* degradability of browse species in sub-humid subtropical savannahs. It was hypothesised that (i) seasonal variations in quality of forage in sub-humid subtropical savannah could affect the degradability of browse species (Winrock, 1992), (ii) natural variations in nutritive value among different plant species may influence the degradability (Meissner *et al.*, 1999; Van Hoven, 2000) and (iii) since tannin may exert negative effects on the nutritive value of forages by reducing intake and digestibility (Kumar & D'Mello, 1995), the addition of tannin-agent such as PEG might reduce these negative effects on digestibility (Makkar *et al.*, 1995a) of tannin-rich species.

5.2 Materials and methods

5.2.1 *In vitro* degradability

The samples used in this chapter are the same samples used in Chapter 4 (*Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata*) which were analyzed for chemical composition. Two experiments were carried out; the first one tested the effect of season and browse species. The second experiment tested the effect of tannins by adding polyethylene glycol 4000 (PEG). Only a small amount of PEG (5.6 mg) was added to each bottle. High amount of PEG (500, 750, 1000 and 1250 mg/g) has been studied by Bueno *et al*. (2008) who found no difference among these levels of PEG. The PEG treatment was applied to samples of the early and the late wet seasons only because the dry season had limited samples due to some species being deciduous.

One gram dry matter was the amount incubated in each Durant bottle. They were subjected to fermentation using an automated technique described by Pell and Schofield (1993) as described in Chapter 4. At the end of 72 h incubation, the terminal pH was measured and bottles content were centrifuged at 18,000 g for 20 minutes at 4 °C. The supernatant after centrifugation was prepared for short chain fatty acids (SCFA) analysis as described by Cottyn and Boucque (1968). The top part of the supernatant was poured into 50 ml flask

and 10 ml of it was pipetted into a 15 ml plastic tube containing 2 ml of 25% metaphosphoric acid and stored in a refrigerator prior to analysis for SCFA; the other part of the supernatant was discarded.

The solid part (R) was dried in fanned oven at 60 ºC for 72 h until constant weight was reached. The difference in weight between incubated material and R was the apparent degradability (ApDeg). The dried solid part was subjected to neutral detergent solution using ANKOM Technology Technique according to Van Soest *et al.* (1991). The difference between the incubated material and neutral detergent fibre (NDF) weight was the true degradability (TrDeg) as described by Van Soest *et al.* (1991) and Blümmel and Becker (1997). Microbial matter (MY) was calculated based on Van Soest (1994) and Blümmel *et al.* (1997b) as the difference between TrDeg and ApDeg. The partitioning factor (PF) and degradation efficiency factor (DEF) for browse species were calculated as follows:

$$PF = TrDeg/GP \quad \text{(Blümmel } et\ al.\text{, 1994; 1997a)}$$

$$DEF = 2PF/T_{1/2} \quad \text{(Ouda \& Nsahlai, 2009)}$$

where: TrDeg = True degradability (mg); GP = Total gas volume (ml); $T_{1/2}$ = time taken to produce $GP_{1/2}$ (h); $GP_{1/2}$ = half of GP (ml).

5.2.2 Short chain fatty acid

For analyses of SCFA, storage samples were thawed and centrifuged at 10,000 g for 10 minutes at 4 °C, 2 ml was filtered through 0.45 µm filter (SUPELCO, USA) into 2 ml new ABC Screw Top Vial. The vials then were loaded into an automatic sampler (HT280, Brescia, Italy) linked to a Gas Chromatograph (YL6100 GC, 600, Young Lin Instrument, Korea) fitted with SGE forte GC capillary column (25m x 0.22mm ID-BP21 0.25µm). The column temperature was programmed at a temperature-cycle from 90 to 120 °C rising by 5 °C. The injector block temperature was maintained at 260°C with nitrogen as the carrier gas (60ml/min). Flow rate of H_2 and air to the detector (flame ionization detector) were 60 and 300 ml/min, respectively. A 1 µl/split 1:80 was auto-injected. The obtained peak of each particular SCFA was calibrated with a standard curve of five different concentrations of pure standard (SIGMA-ALDRICH®).

5.2.3 Statistical analysis

The experiment design was a factorial design for both experiment 1 (5 species x 3 periods) and experiment 2 (5 species x 2 periods x 2 PEG levels). The apparent, true degradability and SCFA raw data were corrected used *Eragrostis* hay data (Appendix 11) by calculating the mean for each season per run, overall mean and then the ratio of the two (the overall mean divided by the mean of each season). The raw data of each parameter were multiplied by the ratio and then subjected to the General Liner Model of SAS (2002) to detemine the effects of season, plant species and PEG on *in vitro* degradabilty; significance was declared at $P<0.05$. Means were compared by least significant difference (LSD). The models used were:

$$Y_{ijk} = \mu + s_i + p_j + (sp)_{ij} + \varepsilon_{ijk} \qquad \text{(Experiment 1);}$$

where, Y_{ij} is the observation, μ is the population mean, s_i is the season effect (i = 1–3), p_j is the plant species effect (j = 1–5), $(sp)_{ij}$ is the interaction between season and plant pecies and ε_{ij} is the residual error.

$$Y_{ijkl} = \mu + s_i + p_j + t_k + (sp)_{ij} + (st)_{ik} + (pt)_{jk} + (spt)_{ijk} + \varepsilon_{ijkl}; \qquad \text{(Experiment 2)}$$

where, Y_{ijk} is the observation, μ is the population mean, s_i is the season effect (i = 1–2), p_j is the plant species effect (j = 1–5), t is polyethelene glycol (PEG) effect (k = 1), $(sp)_{ij}$ is the interaction between season and plant pecies, $(st)_{ik}$ is the interaction between season and PEG, $(pt)_{jk}$ is the interaction between plant pecies and PEG, $(spt)_{ijk}$ is the interaction between season, plant pecies and PEG, and $\varepsilon ijkl$ is the residual error.

5.3 Results

5.3.1 Effects of season and plant species on *in vitro* degradability (Experiment 1)

The effects of season and plant species (experiment 1) on *in vitro* pH, apparent degradability (ApDeg), true degradability (TrDeg), microbial yield (MY), total of short chain fatty acids (SCFA), partitioning factor (PF) and degradation efficiency factor (DEF) are shown in Table 5.1. Season affected ($P<0.001$) ApDeg, TrDeg, MY, PF and DEF, and

also affected (P<0.05) SCFA, and did not affected the pH while browse species affected (P<0.001) all the parameters. The interaction of season x species affected (P<0.001) ApDeg, TrDeg, and DEF and also affected (P<0.05) the PF but did not affect the pH and MY. Among the seasons, ApDeg and TrDeg were higher in the dry season than in the early wet and the late wet seasons while MY was higher in the early wet season than in the dry and the late wet seasons. The SCFA was higher in the late wet season. The PF and DEF were higher in the early wet season than in the dry and the late wet seasons.

Among the five plant species, pH ranged from 6.81 in *A. nilotica* to 6.94 in *S. myrtina*. *Chromolaena odorata* had the highest ApDeg, followed by *A. nilotica* while *A. natalitia*, *D. cinerea* and *S. myrtina* had similar and low ApDeg. *Scutia myrtina* had the highest TrDeg, followed in order by *A. natalitia*, *D. cinerea*, *C. odorata* and *A. nilotica*. *Acacia natalitia* had the highest MY whilst *C. odorata* had the lowest MY. *Chromolaena odorata* had the highest concentration of SCFA, followed by *A. nilotica*, *S. myrtina*, *D. cinerea* and *A. natalitia*. *Acacia natalitia* had the highest PF, whilst *D. cinerea* and *S. myrtina* had similar values and *C. odorata* had the lowest value. *Acacia nilotica* had the lowest DEF (0.99) while the others range from 1.20 to 1.95.

Interaction between season and browse species had different trends for the variables. For *A. natalitia* and *A. nilotica* ApDeg decreased from the dry season to the early and late stages of the wet season. The ApDeg for *D. cinerea* and *S. myrtina* decreased from the dry season to the early wet season from where it increased in the late wet season. *Chromolaena odorata* had similar ApDeg in the dry and the early wet seasons from where it decreased in the late wet season. The TrDeg of *A. natalitia*, *D. cinerea* and *S. myrtina* did not change across the three seasons. For *A. nilotica* and *C. odorata* TrDeg decreased from the dry season to the wet season. The SCFA did not change throughout three seasons in *A. natalitia*, *A. nilotica* and *S. myrtina*, while in *D. cinerea* and *C. odorata* they increased from the dry to the early wet and the late wet seasons.

The PF for *A. natalitia* increased from the dry to the early wet seasons from where it decreased in the late wet season. For *A. nilotica* PF was similar during the three seasons. The PF of *D. cinerea* and *C. odorata* decreased from the dry to the early wet seasons then decreased in the late wet season (*D. cinerea*) or remained similar between the early wet

and the late wet seasons (*C. odorata*). For *S. myrtina* PF increased from the dry to the late wet seasons.

The DEF of *A. natalitia* increased from the dry to the early seasons from where it decreased in the late wet season. For *A. nilotica*, *D. cinerea* and *C. odorata*, DEF decreased from the dry to the early wet seasons then has been similar between the two wet seasons (*A. nilotica*) or decreased (*D. cinerea*) and increased (*C. odorata*) in the wet season. For *S. myrtina* DEF increased from the dry to the late wet seasons.

Within the five plant species and the three seasons, *A. nilotica* had the greatest ApDeg in the dry and the late wet seasons while *C. odorata* had the greatest value during the early wet season. The lowest ApDeg was observed with *A. natalitia* in the dry and the late wet seasons and *S. myrtina* in the early wet season. For TrDeg, *S. myrtina* had the highest value in the dry and late wet seasons while *C. odorata* had the highest value in the early wet season and the lowest value in the dry season and *A. nilotica* the lowest values in the two wet seasons. For MY, *A. natalitia* had highest values during the three seasons. The lowest MY was observed with *C. odorata* in the dry season as well as *A. nilotica* in the early and late wet seasons. For SCFA, *C. odorata* had the greatest concentration in the three seasons followed in decreasing order by *A. nilotica*, *S. myrtina*, *A. natalitia* and *D. cinerea* in the dry and the early wet, except *A. natalitia* in the dry season switched with *D. cinerea* in the early wet season. Whilst the concentration of SCFA in the late wet season followed this decreasing trend: *C. odorata*, *D. cinerea*, *A. nilotica*, *A. natalitia*, and *S. myrtina*. For the PF and DEF, *D. cinerea*, *A. natalitia* and *S. myrtina* had the highest values during the dry, early wet and late wet seasons, respectively, while *C. odorata* had the lowest PF and *A. nilotica* had lowest DEF in the three seasons.

5.3.2 Effects of PEG on *in vitro* degradability (Experiment 2)

Table 5.2 shows the effect of PEG on *in vitro* pH, apparent degradability (ApDeg), true degradability (TrDeg), microbial yield (MY), the total short chain fatty acids (SCFA), partitioning factor (PF) and degradation efficiency factor (DEF) (experiment 2). The addition of PEG affected ($P<0.001$) all the above parameters. Season and its interaction with PEG (Se x PEG) affected ($P<0.001$) the MY, SCFA, PF and DEF while plant species affected ($P<0.001$) all variables (pH, ApDeg, TrDeg, MY, SCFA, PF and DEF). The

interaction between plant species and PEG (Sp x PEG) affected all the above variables except pH while the interaction between season and plant species (Se x Sp), and between season, plant species and PEG (Se x Sp x PEG) affected only the ApDeg, SCFA, PF and DEF.

Table 5.1 Effects of season and plant species on in vitro pH, apparent and true degradability, microbial yield, total of short chain fatty acids, partitioning factor and degradation efficiency factor of plant species harvested from sub-humid subtropical savannah and fermented using rumen fluid (Experiment 1)

Season	Species	pH	ApDeg	TrDeg	MY	SCFA	PF	DEF
Dry	*A. natalitia* [a]	6.88	195	833	640	25.8	27.9	1.50
	A. nilotica	6.78	524	844	321	31.3	17.0	1.17
	D. cinerea	6.97	200	805	605	21. 5	30.7	1.76
	S. myrtina	6.91	223	848	619	28.0	19.1	1.27
	C. odorata [b]	6.81	530	734	210	50.2	9.7	1.40
Early	*A. natalitia*	6.90	175	809	636	16.7	35.9	3.05
Wet	*A. nilotica*	6.85	400	681	286	29.6	16.8	0.89
	D. cinerea	6.88	169	789	630	26.5	27.2	1.63
	S. myrtina	6.94	176	815	635	23.4	-	-
	C. odorata	6.80	521	845	324	57.2	07.1	0.99
Late	*A. natalitia*	6.86	174	836	662	25.4	19.9	1.30
Wet	*A. nilotica*	6.81	421	680	315	28.1	15.8	0.92
	D. cinerea	6.84	227	778	551	28.9	15.9	1.27
	S. myrtina	6.92	212	862	650	25.3	30.1	1.47
	C. odorata	6.79	366	718	350	55.6	7.0	1.21
	P <	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	RMSE	0.239	49.12	71.65	86.14	2.092	6.766	0.112
	LSD	0.062	12.78	19.02	6.2011	1.124	2.841	0.086
Sources of variation effects								25.8
	Season	ns	***	***	***	*	***	***
	Species	***	***	***	***	***	***	***
	Interaction	ns	***	***	ns	***	*	***

[a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; ApDeg - the apparent degradability (g kg^{-1} DM); TrDeg – true degradability (g kg^{-1} DM); MY – microbial yield (g kg^{-1} DM); SCFA – total of short chain fatty acids (mmol L^{-1}); PF – portioning factor; DEF=degradation efficiency factor; RMSE - root mean square error; ns (P>0.05), * (P<05); *** (P<0.001).

With the addition of PEG, the ApDeg increased during the two seasons (early and late wet) with two exceptions: *C. odorata* and *A. nilotica* decreased during the early wet and the late wet seasons, respectively. The five plant species decreased in TrDeg, MY, PF and DEF during the early wet and the late wet seasons. However, with regards to TrDeg, *A. nilotica* and *C. odorata* increased during the early wet and the late wet season, respectively. The addition of PEG resulted in a higher increase in concentration of SCFA during the early

wet than the late wet seasons. In the early wet season, *S. myrtina* had a high increase in SCFA followed by *A. natalitia*, *A. nilotica* and *D. cinerea*, while *C. odorata* had a slight decrease. The increasing in SCFA during the late wet season followed this order: *S. myrtina*, *A. nilotica*, *A. natalitia*, *D. cinerea* and *C. odorata*.

Table 5.2 Effects of PEG on *in vitro* apparent and true degradability, microbial yield, total of short chain fatty acids, partitioning factor and degradation efficiency factor of browse species (Sp) samples in two seasons (Se) at Zululand Thornveld and fermented using rumen fluid (Experiment 2)

Se	Sp	PEG	pH	ApDeg	TrDeg	MY	SCFA	PF	DEF
Early	*Ana* [a]	-	6.92	173	821	652	17.7	34.7	3.6
wet	*Ana*	+	6.80	194	625	440	36.0	8.2	0.81
	An	-	6.86	408	688	291	28.1	16.7	0.88
	An	+	6.70	436	710	274	45.7	8	0.71
	Dc	-	6.87	166	793	625	24.7	25.3	1.57
	Dc	+	6.84	249	691	442	37.5	9.7	1.05
	Sm	-	6.97	171	816	649	25.8	-	-
	Sm	+	6.81	294	647	353	45.3	7.2	0.68
	Co [b]	-	6.83	532	738	209	56.2	6.7	0.94
	Co	+	6.78	510	728	218	50.4	6.4	0.88
Late	*Ana*	-	6.86	163	831	665	27.3	17.9	1.22
wet	*Ana*	+	6.82	224	678	451	33.0	9.8	1.14
	An	-	6.78	392	684	300	29.1	15	0.9
	An	+	6.72	383	643	257	35.6	8.2	0.75
	Dc	-	6.83	220	782	562	30.6	16.5	1.3
	Dc	+	6.81	280	695	415	35.0	9.3	1.18
	Sm	-	6.94	210	856	657	24.1	27.2	1.2
	Sm	+	6.78	287	696	414	44.1	7.9	0.72
	Co	-	6.77	369	710	331	54.5	7.2	1.39
	Co	+	6.76	529	725	196	56.1	7.0	1.00
	P <		0.001	0.001	0.001	0.001	0.001	0.001	0.001
	RMSE		0.206	59.57	76.12	74.72	2.700	1.554	0.278
	LSD		0.051	14.79	18.9	18.55	1.254	0.438	0.078
Sources of variation effects									
	Se		ns	ns	ns	***	ns	***	*
	Sp		***	***	**	***	***	***	***
	PEG		*	***	***	***	***	***	***
	Se x Sp		ns	*	ns	ns	***	***	***
	Se x PEG		ns	ns	ns	***	***	***	***
	Sp x PEG		ns	**	***	***	***	***	***
	Se x Sp x PEG		ns	***	ns	ns	***	***	***

Ana – Acacia natalitia; *An – Acacia nilotica*; *Dc – Dichrostachys cinerea*; *Sm – Scutia myrtina*; *Co – Chromolaena odorata*; [a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; ApDeg - the apparent degradability (g kg^{-1} DM); TrDeg – true degradability (g kg^{-1} DM); MY – microbial yield (g kg^{-1} DM); SCFA – total of short chain fatty acids (mmol L^{-1}); PF – portioning factor; DEF=degradation efficiency factor; LSD – least significant difference; RMSE - root mean square error; ns (P>0.05), * (P<05); ** (P<0.1); *** (P<0.001).

5.4 Discussion

Seasonality was hypothesised to influence feed value whereby climatic factors are associated with changes in nutritive value and availability. The results supported the hypothesis. The true degradability (TrDeg) across the seasons were within the range of 900–540 g/kg DM reported by Yayneshet *et al.* (2009) who studied the *in vitro* DM digestibility for browse species in Tembien, Ethiopia, while overlapping reported ranges for other browse species at Wukro-Ethiopia (Yayneshet *et al.,* 2009), North-Eastern Nigeria (Njidda & Nasiru, 2010) and Egypt (Sallam *et al.,* 2010). These changes in the nutritive values of browse species could be due to differences in chemical composition caused by fluctuations in climatic conditions between seasons, and their influences on cell wall lignification (Larbi *et al.*, 1996). Moreover, the nutritive values of different plants in tropical areas have been evaluated using the *in vitro* technique (Nsahlai *et al.*, 1994; Yayneshet *et al.*, 2009; Njidda & Nasiru, 2010; Sallam *et al.*, 2010). The TrDeg was higher during the dry season than either the early or late stage of the wet season, which may be related to the fact that plant leaves in the early wet season had high CT and high CP, while in the late wet season the leaves had higher NDF level (Basha *et al*., 2012). This may explain why the TrDeg was low in the two wet stages. Reed *et al.* (1990) reported that tannins are present in the fibres (NDF and ADF) and are bound to the cell wall and protein and appear to be responsible for decreasing digestibility. It has been reported that the decrease in the digestibility of dietary fibres because of the presence of anti-nutritional factors present in the feed, affect fibrolytic micro-organisms (Reed, 1995; Ngwa *et al*., 2001). Furthermore, seasonal differences in *in vitro* digestibility are mainly related to the chemical composition of the samples, particularly to their cell wall content. The cell wall fraction could have a negative effect on browse digestibility (Wilson, 1977; Mertens, 1993). For CP, the observed variation in TrDeg may be due to complexes formed between tannins and proteins, which were mostly insoluble in the neutral detergent and could add to the undegradable fraction (Makkar *et al.*, 1995a, b).

The results of microbial yield need some explanation because of the large variation in the result of apparent degradability and microbial yield among species and among seasons. *In vitro* apparent degradability is associated with microbial yield because the estimation of MY resulted from the differences between ApDeg and TrDeg (Van Soest, 1994; Blümmel & Becker, 1997). The ApDeg for some browse species (*A. natalitia, D. cinerea* and *S.*

myrtina) appeared unreasonably low, resulting to very high MY; and reasonable MY values for others (*A. nilotica* and *C. odorata*). When substrate is fermented *in vitro* or *in vivo*, the digested matter is measured principally as SCFA, microbial cell, CO_2 and CH_4. This fermentation process results in loss of mass of incubated substrate which could be reflected in the apparent digestibility and reveal the level of fermentation and end-products produced. For all species during the three seasons, the range of gas production was 26–104 ml g^{-1} DM (Chapter 4) which range from poor to good and confirmed the loss of mass due to the fermentation process. Thus, because values of MY were higher than the physiological limit (200-260 g kg^{-1} DOM, Bucholtz & Bergen, 1973), the high value of MY for some browse species may be attributed to some reasons not measured in this study such as total soluble material hydrolysed but not fermented. Thus, the discussion is restricted to the MY in *A. nilotica* and *C. odorata.* Their range was within the range reported by Ranilla *et al.* (2001) and Ouda and Nsahlai (2007) who used *in vitro* technique.

Microbial yield increased with CP and decreased with condensed tannins and fibre concentration. However, these chemical components were varied among the three seasons of this study (Basha *et al*., 2012). It has been reported that anti-nutritional factors might influence efficiency of microbial synthesis (Reed, 1995; Ben Salem *et al*., 1997b; Kaitho *et al*., 1998a). For *A. nilotica,* the MY was low in the early wet season that may be due to the high tannins or NDF contents. The leaves of *A. nilotica* have a high level of condensed tannins and NDF in the early wet season compared to the dry and late wet seasons (Basha *et al*., 2012). This is in agreement with Guimarães-Beelen *et al*. (2006) who reported that the activities of micro-organisms were significantly lower in the tannin-rich treatment compared to the low tannin treatment. Moreover, tannins have been reported to be toxic to rumen micro-organisms and so limiting the degradability of cell walls (Jung, 1988). In addition, Ngwa *et al*. (2001) suggested that the variation in degradability for lucerne and maize stover may refer to the variation in their cell wall components. Nevertheless, for *C. odorata*, the MY has shown an increasing order which corresponding with what Basha *et al*. (2012) found for CP contents among the three seasons. This result supports a report that DM digestibility was positively related to CP content (Minson, 1982). Additionally, the use of protein by ruminants is controlled by ruminal protein degradation (Pathak, 2008). The process of ruminal protein degradation results in released nitrogen compounds which are important for microbial growth (Pathak, 2008). However, in the browse species, condensed tannins may bind with protein and make it unavailable for rumen microbes

while *C. odorata* was free of condensed tannins (0.5 g kg^{-1} DM) (Basha *et al.*, 2012). On the other hand, the carbohydrates (CHO) were the main energy resources for rumen microbes. The synchronization between release of energy from dietary fibrous and nitrogen from dietary protein may further improve nitrogen utilization (Salter *et al.*, 1979; Herrera-Saldana *et al.*, 1990). However, gases are mainly the outcome of carbohydrates fermented to SCFA (Wolin, 1960). The SCFA results were varied among the three seasons that may be due to the natural difference among the species. Beuvink and Spoelstra (1992) reported that the molar proportion of individual SCFA produced is dependent on the type of substrate. Fermentation of tannin-rich plant species produced less SCFA than those with low tannin plant species (*C. odorata*). Tannins reduce the production of SCFA in tannin-rich feeds compared to tannin-low feeds (Mbugua *et al.*, 2008).

To compare the wide variation of the partitioning factor (PF) results to the pervious results; firstly, some explanations will take place. In theory, the PF for tannin-free plants differs from 2.75 to 4.41 mg ml^{-1} equivalent 10 to 32 mg of Y_{ATP} (adenosine triphosphate yield) which is assumed to be microbial efficiency (Blümmel *et al.*, 1997a). Makkar *et al.* (1997a) and Blümmel *et al.* (2005) assumed that a PF value that is higher than the theoretical maximum value (4.41 mg ml^{-1}) is not a reasonable biological value. In this study, PF values for the five browse plants were higher (7.0 ─ 35.9 mg ml^{-1}) than the theoretical upper limit value. Blümmel *et al.* (2005) and Ouda and Nsahlai (2007; 2009) without correcting gas released from buffers reported PF values within the current range. Baba *et al.* (2002) reported that PF for browses ranged from 5.05 to 11.05. Makkar *et al.* (1997a) suggested that the estimation of PF for many browse species may not work properly, because of overestimation of the degradability values due to the undegradable substrate fraction, such as tannin complexes in the residual. However, Baba *et al.* (2002) assumed that the use of PF values for comparing browse species (tannin-rich plants) is useful. Thus, the PF level was generally low in the late wet season that may be attributed to the low values of TrDeg in this season and in the same time the maximum gas production was varied among the three seasons, since the PF calculated as the ratio between TrDeg and gas production. The increase in gas production might result in lower PF to microbial yield (Makkar *et al.*, 1998).

The results of DEF in the current study were varied among seasons which overlapped reported values by Ouda and Nsahlai (2009) who studied *Sercea lespedeza* rations in *in*

vitro fermentation technique. They reported that increase *S. lespedeza* ratio in ration resulted in decreasing DEF that may be due to the CT. Ouda and Nsahlai (2009) suggested that is important to calculate the DEF when feeds are evaluated using *in vitro* gas production technique. Because the time required to produce half the maximum gas ($T_{1/2}$) (measured in Chapter 4) could affect the DEF values, the long $T_{1/2}$ gives low DEF values which represent low nutritive value and microbial efficiency. Furthermore, Cone and Van Gelder (2000) found positive relationship between fermentation rates of substrate and microbial efficiency.

Plant species was hypothesised to influence feed value whereby natural variations in chemical composition among different plant species. The results supported the hypothesis. With respect to the pH, *S. myrtina* had the highest levels while *C. odorata* has the lowest levels across the three seasons that may be attributed to the high tannins level in *S. myrtnia* (Basha *et al*., 2012). Zimmer and Cordesse (1996) recorded high pH level in the tannin-rich feed compared to the tannin-free feed. The five browse species leaves had variable TrDeg, SCFA, PF and DEF that may be due to variations in chemical constituents among the browse species (Scogings *et al*., 2004; Basha *et al*., 2009; 2012; Lebopa *et al*., 2011). *In vitro* dry matter digestibility varies widely among woody tree and shrub species and ranged from 380 to 780 g kg^{-1} DM (Wilson, 1977; Skarpe & Bergstörm, 1986). The high value of TrDeg in *S. myrtnia* overlapped this range. The low TrDeg in *A. nilotica* was higher than the range and higher than what Madibela *et al.* (2006) recorded *in vitro* for *A. nilotica*. The high production of SCFA in *C. odorata* and low in *A. natalitia* may be due to the high CP and lower CT in *C. odorata* and vice versa for *A. natalitia*. Mbugua *et al.* (2008) reported that tannins may decrease the production of SCFA in tannin-rich feeds. The high PF in *A. natalitia* and low in *C. odorata* attributed to the high and low TrDeg in *A. natalitia* and *C. odorata* as well as to low and high gas production (Chapter 4), respectively, because the PF was calculated as the ratio between TrDeg and gas production.

The addition of PEG was hypothesised to reduce the negative effects of tannin on *in vitro* digestibility exactly for of tannin-rich species. Generally, adding of PEG slightly decreased the pH in browse species suggesting that PEG binds with tannins. This is consistent with findings of Zimmer and Cordesse (1996) in an *in vivo* trial that the minimum range of pH was 6.3-6.5 in the tannin-rich feed compared with 6.0 in the tannin-free feed. In the current

study, the pH values with added PEG were 6.70–6.84 which falls within the normal range of pH in the rumen (5.5-7.0) (Jones & Mangan, 1977). Makkar *et al.* (1995a) pointed out that for the *in vitro* gas method, at the starting point the pH was 6.80-7.00 and at the end of 24 h incubation was 6.70-6.85. Adding of PEG decreased the true degradability (TrDeg) for plant species except for *A. nilotica* and *C. odorata*. These results were not supported by the hypothesis except for *A. nilotica*. Makkar *et al.* (1995a) reported that the addition of PEG decrease the true degradability due to the occurrence of tannin-PEG complexes in the residues. These complexes are partly or completely insoluble in boiling water, neutral detergent solution, acid detergent solution and other organic solvents (Jones & Mangan, 1977; Makkar *et al.*, 1995a). The increase of TrDeg in *A. nilotica* may be due to type of the tannins. Jones and Palmer (2000) reported the incubation of PEG with tannin-rich diets *in vitro* may react differently with all diets because of differences in tannin chemistry in diets. Moreover, the reaction of PEG and tannin depend on the nature and the level of the secondary compounds, particularly tannins (Ebong, 1995). The non significant change in the TrDeg for *C. odorata* may be due to lack or less reaction of PEG with the components or soluble complexes may form between PEG and other components.

In the current study, microbial yield for tannin-rich browse species increased in response to PEG treatment. These results supported the hypothesis. That may be attributed to release of protein as the result of bound PEG with tannins. Reduced protein degradation in the rumen is one of the major causes for inefficient utilization of protein in ruminants (Pathak, 2008). Thus, the addition of PEG increased the availability of protein to rumen micro-organisms by releasing protein from tannin-protein complexes (Makkar *et al.*, 1995a). The results in current study support the finding of Ben Salem *et al.* (1999) and (2000). Contrary, Makkar *et al.* (1998) reported that addition of PEG reduced the efficiency of microbial protein synthesis. The addition of PEG reduced the fermentation efficiency as confirmed by the reducing partitioning factors (Mlambo *et al.*, 2008; 2009). This finding is in agreement with the results of Baba *et al.* (2002) and Mlambo *et al.* (2009) who reported that addition of PEG effectively depressed partitioning factors. So the decrease in PF as response to addition of PEG in this study could be ascribed to the increase in gas production. Fermentation of tannin-rich browse samples produced less SCFA than *C. odorata* (low tannin), moreover, addition of PEG maximized SCFA production in tannin-rich browses, which supported the hypothesis. Similar findings *in vitro* have been reported of the effect of tannins on SCFA production (McSweeney *et al.*, 1999; Getachew *et al.*, 2000b; Mbugua

et al., 2008). Mbugua *et al.* (2008) confirmed that incubation of PEG with tannin-rich samples led to increase in SCFA production. Furthermore, *in vivo* single SCFA, particularly acetate was poor in tannin-fed sheep compared to those incubated with PEG (Waghorn *et al.*, 1994b).

5.5 Conclusions

In vitro degradability parameters varied among seasons and among different browse species harvested from sub-humid subtropical savannah of KwaZulu-Natal Province, South Africa, during the dry, the early, and the late wet seasons. The addition of PEG (tannin binding agent) emphasizes inhibitory effect of tannins on rumen microbial activity. The results suggest that to use these browse species efficiently, there is need to reduce the effect of condensed tannins.

Chapter 6

Effects of season and browse species on *in sacco* degradability of forages in the sub-humid subtropical savannah, South Africa*

Abstract

Effect of season and plant species on *in sacco* dry matter (DM) and crude protein (CP) degradability of five plant species from sub-humid subtropical savannah of South Africa were investigated. The plant species were *Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata*. Leaves were harvested during the dry (June/July), early wet (November/December) and late wet (February/March) seasons and subjected to degradation using the nylon bags technique carried out in three replicates. Season affected the potential DM degradability and effective degradation of both DM and CP. Browse species affected all parameters except the slowly degradable fraction of CP. Interaction between season and browse species affected all parameters except the potential and slowly degradable fraction of CP. *Chromolaena odorata* had the highest estimated parameters of degradation during the three seasons compared to the other browse species. Based on potential and effective degradation, the plant species followed this decreasing order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. All parameters were positively correlated to CP except the soluble and slowly degradable fractions of DM and CP, respectively, and lag time of both DM and CP. All parameters were negatively correlated to neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) except the slowly degradable fraction and rate of degradation. Condensed tannins (CT) were negatively correlated with all parameters except the slowly degradable fraction and potential degradation of DM and CP, and lag time of DM degradation.

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* Basha, N.A.D., Scogings, P.F. and Nsahlai, I.V., 2011. Effects of season and species on *in sacco* degradability of plant species in the sub-humid subtropical savannah, South Africa. In: Proceedings of the 8th International Symposium on the Nutrition of Herbivores, Aberystwyth, Wales Uk, 6-9 September 2011, Cambridge University Press, pp 487.

Cellulose was negatively correlated with soluble fraction of DM and lag time of CP degradation. The results suggest that these plant species have potential to be used as feed supplements. *Chromolaena odorata* has the highest potential as feed protein source in ruminants. We concluded that season and species affected *in sacco* degradability of DM and CP of browse species.

Key words: *Chromolaena odorata,* nutritive value, dry matter degradability, crude protein, Condensed tannins.

6.1 Introduction

Smallholder farmers in subtropical savannah of Africa keep different ruminant species, most of which survive on natural pastures (Aganga & Tshwenyane, 2003). The productivity of these ruminant species depends on quantity and quality of the feeds (forage), which is affected by seasonal fluctuations (Winrock, 1992). The lowest quantity of forage occurs during the dry season and may limit feeding and production of livestock. One strategy to increase value is the use of trees and shrubs as a sufficient source of food for ruminants. Some of the forages are legumes, and legumes offer important sources of protein to maintain ruminant production in tropical savannah (Ngwa *et al.*, 2003). Browse and shrub fodders are essential because they reduce the seasonal limitation in ruminant feed (Topps, 1992). However, the distribution of tannins and other phenolic compounds in shrubs and tree leaves limits their utilization as animal feed (Tolera *et al.*, 1997). Hence, the evaluation of nutritive value of browse trees becomes important only when the browse species is used as ruminant feeds.

In sacco degradability is a main evaluation technique of nutritive value of forages (Ørskov & McDonald, 1979). It is a useful method for ranking browse trees in terms of quality (Mehrez & Ørskov, 1977) and for evaluating the digestive abilities of ruminant species (Migongo-Bake, 1992). *In sacco* estimation has a benefit of estimating the degradation of particular constituent of feed such as dry matter (DM), crude protein (CP) and other constituents. Additionally, *in sacco* technique does not only determine the extent of degradation but also the part that degrades fast and its rate (Ørskov & McDonald, 1979). Moreover, estimation of the soluble and slowly degradable fractions is necessary for

dietary protein. This estimation may allow evaluating the contribution of dietary protein to the rumen microbial, and may allow estimating the quantity of protein that could bypass the rumen and become accessible for digestion in the small intestine to the host animal. This is helpful for assessment of tropical browse trees as protein source.

The aim of this chapter was to determine the effect of season and plant species on *in sacco* degradation characteristics of dry matter and nitrogen on edible forage of browse species in sub-humid subtropical savannah. It was predicated that (i) changes in quality of forage due to seasonal variations in savannah may affect their rumen degradability (Larbi *et al*., 1998; Ramírez-Orduña *et al*., 2003; Camacho *et al*., 2010) and (ii) inherent differences in nutritional value among plant species could influence the rumen degradability (Nsahlai *et al*., 1994; Ndlovu & Nherera, 1997) which might relate to their chemicals traits (Nsahlai *et al*., 1994; Ndlovu & Nherera, 1997; Apori *et al*., 1998; Larbi *et al*., 1998).

6.2 Materials and methods

6.2.1 Plant samples and experimental animals

The samples used in this chapter are the same samples used in Chapter (4) and (5) which were analyzed for chemical composition in Chapter (4), but here the dried samples were milled through a 2-mm mesh sieve (Retsch GmbH & Co. KG 5657 HANN 1, West-Germany).

The experiment was conducted at the Livestock Section of the University of KwaZulu-Natal Research Farm (Ukulinga), South Africa. Three rumen-fistulated cows (average weight: 350 ± 45 kg LW) were used. The cows were each fed 2 kg of Lucerne hay per day, with *ad libitum* access to hay, water and a mineral lick. The cows were adapted to the diet for one week before being used in the experiments. The experiment followed the nylon bag technique described by Mehrez and Ørskov (1977).

6.2.2 *In sacco* degradability

Three grams of each dry sample per incubation period replicated thrice (three nylon bags) were incubated for 0, 3, 6, 9, 12, 24, 48 and 72 hours in three fistulated cows (one

bag/cow). The whole bag size was 18x8 cm with pore size of 40-60 µm. Samples were incubated in four batches; all the samples within a batch were withdrawn simultaneously (Table 6.1). The withdrawn samples were cleaned with water and kept in a refrigerator till washing date, when all samples were washed together including zero hour ones. Washing occurred in a semi–automatic washing machine (Hoovermatic model T4350, South Africa) for 6 times in cycles of 5 min. The washed bags were dried in an oven (LABCO, model 5SOE1B, P.O. Box 155, Maraisburg 1700) at 60˚C for 48 h, cooled in a desiccator and weighed. Residues were analyzed for nitrogen by AOAC method 990.03 (AOAC, 1997) using a LECO, FP2000, nitrogen analyzer. The DM and CP degradation data were fitted to the exponential equation:

$$Y = a + b\,(1 - e^{-c(t-lt)}) \quad \text{(McDonald, 1981);}$$

where, Y is the degradability of DM and CP at time (t), a is the soluble fraction which is rapidly washed out of the bags, b is the insoluble fraction which is potentially degradable by micro-organisms, c is the degradation rate of fraction b per hour and lt is the lag time.

The effective degradability (ED) of DM and CP were calculated at a rumen out flow rate (r) of 0.03 h^{-1} using the following equation:

$$ED = a + b*c/(c+r).$$

6.2.3 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS (2002) in a three seasons × 5 feeds factorial design with three replicates. The model used was:

$$Y_{ijk} = \mu + s_i + p_j + (sp)_{ij} + \varepsilon_{ijk};$$

where, Y_{ijk} is the observation, µ is the population mean, s_i is the season effect (i = 1–3), p_j is the plant species effect (j = 1–5), $(sp)_{ij}$ is the interaction between season and plant pecies and ε_{ijk} is the residual error. Statistical significance was declared at $P<0.05$. Means were compared by least significant difference (LSD). Correlation was used to test the relationships between *in sacco* degradability and chemical variables of browse species.

Table 6.1 Incubation and withdrawal program for five samples of plant species in fistulated cattle using the *in sacco* method (Mehrez & Ørskov, 1977)

	Incubation sample details				Withdraw	
Group	Day	period	time	total bags	day	time
1st	Day 1	6-hour	8:00	45	Day 1	14:00
	Day 1	3-hour	11:00	45	Day 1	14:00
2nd	Day 2	12-hour	8:00	45	Day 2	20:00
	Day 2	9-hour	11:00	45	Day 2	20:00
3rd	Day 3	72-hour	8:00	45	Day 6	8:00
	Day 4	36-hour	20:00	45	Day 6	8:00
4th	Day 7	48-hour	8:00	45	Day 9	8:00
	Day 8	24-hour	8:00	45	Day 9	8:00
	Day 9	0-hour (45 bags) + all other samples			washing	

6.3 Results

6.3.1 Effects of season and plant species on *in sacco* dry matter degradation

Table 6.2 shows the effect of season and plant species on *in sacco* dry matter degradation. Season affected (P<0.001) the soluble fraction (a_{dm}), potential degradability (PD_{dm}), effective dry matter degradability (ED_{dm}) and lag time (lt_{dm}). While browse species and its interaction with season affected (P<0.001) all variables (a_{dm}, b_{dm}, c_{dm}, PD_{dm}, ED_{dm} and lt_{dm}). Among the three seasons, the soluble fraction was higher in the dry season than in the early wet and the late wet seasons. The PD_{dm} and ED_{dm} were higher in the dry season than in the early wet and the late wet seasons. The lt_{dm} was longest in the late wet season and shortest in the dry season.

Among the five plant species, *A. natalitia* had the highest soluble fraction (a_{dm}) and *C. odorata* had moderate a_{dm}, while *A. nilotica.*, *D. cinerea* and *S. myrtina* had similar a_{dm}. *Chromo*laenea *odorata* had the highest insoluble degradability (b_{dm}), while *A. natalitia* and *S. myrtina* had similar and moderate b_{dm}, and *A. nilotica* and *D. cinerea* had the lowest b_{dm}. *Chromo*laenea *odorata* had the fastest degradation rate, followed by *A. natalitia*, *A. nilotica*, *D. cinerea* and *S. myrtina* in this order. The PD_{dm} and ED_{dm} showed similar trend among the species, *C. odorata* had the highest values followed by *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea* in this order. *Acacia natalitia* had the longest lt_{dm}, whilst *S. myrtina* had the shortest lt_{dm}.

Table 6.2 *In sacco* dry matter degradation constants of plant species harvested at different three seasons from sub-humid subtropical savannah, South Africa

Season (Se)	Species (Sp)	a_{dm} (g kg^{-1})	b_{dm} (g kg^{-1})	c_{dm} (h^{-1})	$PDdm$ (g kg^{-1})	ED_{dm} (g kg^{-1})	lt_{dm} (h)
Dry	*A. natalitia* [a]	309	521	0.0317	829	576	-1.26
	A. nilotica	528	360	0.0655	887	774	0.85
	D. cinerea	307	289	0.0257	597	430	-0.38
	S. myrtina	292	519	0.0223	812	493	-2.10
	C. odorata [b]	353	484	0.3411	837	796	-0.06
Early wet	*A. natalitia*	268	451	0.0354	719	511	-0.24
	A. nilotica	500	357	0.0657	857	745	1.00
	D. cinerea	331	227	0.0400	559	458	1.05
	S. myrtina	242	449	0.0203	690	423	-0.57
	C. odorata	329	609	0.2803	938	879	0.06
Late wet	*A. natalitia*	208	391	0.0420	598	434	0.24
	A. nilotica	327	443	0.0882	771	658	-0.26
	D. cinerea	222	324	0.0394	546	394	0.54
	S. myrtina	255	399	0.0205	654	409	0.83
	C. odorata	317	632	0.2292	949	876	-0.03
	P<	0.001	0.001	0.001	0.001	0.001	0.001
	RMSE	7.110	50.930	0.029	50.160	10.630	0.648
	LSD	6.863	49.181	0.028	48.432	10.270	0.626
Sources of variation effects							
	Se	***	ns	ns	***	***	**
	Sp	***	***	***	***	***	**
	Se x SP	***	***	*	***	***	**

[a] Formerly part of Acacia karroo (Coates Palgrave, 2002); [b] invasive non-native species; a_{dm} - the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable; b_{dm} - the proportion of insoluble nutrient which is potentially degradable by micro-organisms; c_{dm} - the degradation rate of fraction b_{dm} per hour; PD_{dm} – the potential degradability; ED_{dm} - effective dry matter degradability; lt_{dm} – lag time; LSD – least significant difference; RMSE - root mean square error; ns (P>0.05), * (P<05); *** (P<0.001).

Interaction between season and browse species showed different trends for the variables. For the five species, except *D. cinerea*, soluble fraction decreased from the dry to the early wet seasons then decreased in the late wet season (*A. natalitia*, *A. nilotica* and *C. odorata*) or increased in the late wet season (*S. myrtina*), while the soluble fraction of *D. cinerea* increased from the dry to the early wet seasons from where it decreased in the late wet season. The degradation rate (c_{dm}) of all five plant species except *C. odorata* were low and did not change throughout the three seasons, while degradation rates of *C. odorata* decreased from the dry to the early wet and late wet seasons in this order.

The PD_{dm} for *A. natalitia* decreased from the dry season to the early wet and the late wet seasons in this order. For *A. nilotica*, the PD_{dm} was similar between the dry and the early wet seasons but decreased in the late wet season. The PD_{dm} of *D. cinerea* did not change during the three seasons. For *S. myrtina* and *C. odorata*, the PD_{dm} decreased (*S. myrtina*) or increased (*C. odorata*) from the dry to the early wet and late wet seasons which were similar.

The ED_{dm} for *A. natalitia*, *A. nilotica* and *S. myrtina* decreased from the dry to the early wet and the late wet seasons in this order. For *D. cinerea* and *C. odorata* the ED_{dm} increased from the dry to the early wet seasons then decreased in the late wet season (*D. cinerea*) or remained similar between the early wet and the late wet season (*C. odorata*).

The lag time (lt_{dm}) for *A. natalitia* and *S. myrtina* increased from the dry to the early wet and late wet seasons in this order. For *D. cinerea* and *C. odorata* the lt_{dm} increased from the dry to the early wet seasons from where it decreased in the late wet season.

6.3.2 Effects of season and plant species on *in sacco* nitrogen degradation

Table 6.3 shows the effect of season and plant species on *in sacco* nitrogen degradation. Season affected ($P<0.001$) only the soluble fraction degradability (a_n) and ED_n. Plant species strongly affected ($P<0.001$) a_n, c_n, PD_n and ED_n, weakly affected ($P<0.05$) the lt_n. Interaction between season and palnt species affected ($P<0.001$) a_n, c_n and ED_n. Among the three seasons, a_n and ED_n were higher in the early wet season than in the dry and the late wet seasons.

Among the five plant species, *C. odorata* had the highest soluble fraction (a_n), while *A. nilotica* and *S. myrtina* had moderate (a_n), and *A. natalitia* and *D. cinerea* had similar and low a_n. *Chromo*laenea *odorata* had the fastest degradation rate (c_n) followed by *A. nilotica*, *A. natalitia*, *D. cinerea* and *S. myrtina* in this order. *Chromo*laenea *odorata* had the highest PD_n and ED_n followed by *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea* in this order. *Acacia nilotica* had the longest lt_n, whilst *S. myrtina* had the shortest lt_n.

According to interaction between season and browse species, the a_n fraction in *A. natalitia*, *S. myrtina* and *C. odorata* decreased from the dry to the early wet seasons which was either

similar to the late wet season (*A. natalitia* and *S. myrtina*) or decreased in the late wet season (*C. odorata*). For *A. natalitia,* a_n decreased from the dry (268 g kg^{-1}) to the early wet (232 g kg^{-1}) seasons, then either increased in the late wet (242 g kg^{-1}) season (*A. natalitia)* or remained similar to the late wet seasons (*D. cinerea*). For *D. cinerea*, a_n increased from the dry (172 g kg^{-1}) to the early wet (276 g kg^{-1}) seasons and then decreased in the late wet (261 g kg^{-1}) season.

During the dry season, the degradation rate (c_n) was slowest with *D. cinerea*, *A. natalitia* and *S. myrtina;* intermediate with *A. nilotica* and fastest *C. odorata.* During the early wet season, the degradation rate was slowest for *A. natalitia* and *S. myrtina*; intermediate for *A. nilotica* and *D. cinerea*; and fastest for *C. odorata.* For the late wet season, the degradation rate was fastest for *C. odorata*; fast for *A. nilotica*; intermediate and similar for *A. natalitia* and *D. cinerea*, and slowest for *S. myrtina*. Among seasons, *A. nilotica* for which the degradation rates were similar between the dry and the early wet seasons but higher during the late wet season. The degradation rate of *D. cinerea* was fastest during the early wet season and slowest during the dry season. For *C. odorata,* the degradation rate was fastest, moderate and low during the dry, the early wet and the late wet seasons, respectively.

The effective nitrogen degradability (ED_n) for *A. natalitia* and *S. myrtina* decreased from the dry to the early wet seasons from where it remained similar (*A. natalitia*) or decreased in the late wet season (*S. myrtina*) For *A. nilotica*, *D. cinerea* and *C. odorata* the ED_n increased from the dry to the early wet seasons then decreased in the late wet season (*A. nilotica* and *C. odorata*) or remained similar between the early wet and the late wet seasons (*D. cinerea*).

6.3.3 Correlation between chemical composition and *in sacco* degradability

Table 6.4 shows the correlations between the CP, NDF, ADF, ADL, CT, cellulose (Cell) and hemicellulose (Hcell), and DM and nitrogen degradation parameters. Crude protein was strongly and positively correlated ($P<0.001$) with degradation rate (c_{dm} and c_n), which was negatively correlated ($P<0.05$) to CT. Crude protein was strongly and positively correlated ($P<0.001$) with ED_n and moderately and positively correlated ($P<0.01$) with ED_{dm} and a_n. Crude protein was weakly and positively correlated ($P<0.05$) with b_{dm}, PD_{dm} and PD_n. The soluble fraction (a_{dm}) was strongly and negatively correlated ($P<0.001$) to

fibre fractions (NDF, ADF and ADL) while a_n was moderately and negatively correlated (P<0.01) to NDF and ADL as well as a_n was weakly correlated (P<0.05) to ADF. Neutral detergent fibre was strongly and negatively correlated (P<0.001) with PD_{dm} and ED_{dm} which were moderately correlated (P<0.01) with ADF and ADL. Effective N degradation (ED_n) and lt_n had negative correlations (P<0.01) with NDF; and weak negative correlations (P<0.05) with ADF, ADL and CT. The PD_n was negative correlated (P<0.05) to NDF. Condensed tannins had negative correlation (P<0.05) with a_{dm}, a_n and ED_{dm}. Cellulose was negatively correlated (P<0.05) with a_{dm} and lt_n.

Table 6.3 *In sacco* nitrogen degradation constants of plant species harvested at three seasons harvested at different three seasons from sub-humid subtropical savannah, South Africa

Season (Se)	Species (Sp)	a_n (g kg^{-1})	b_n (g kg^{-1})	c_n (h^{-1})	PD_n (g kg^{-1})	ED_n (g kg^{-1})	lt_n (h)
Dry	*A. natalitia* [a]	268	646	0.0297	914	570	0.53
	A. nilotica	351	522	0.0569	873	692	1.65
	D. cinerea	172	543	0.0141	715	338	0.71
	S. myrtina	336	450	0.0326	786	536	-0.09
	C. odorata [b]	434	481	0.2822	914	868	-0.07
Early wet	*A. natalitia*	232	545	0.0359	776	526	-0.75
	A. nilotica	432	452	0.0538	885	722	1.69
	D. cinerea	276	256	0.0562	532	443	-0.89
	S. myrtina	287	432	0.0262	719	464	-0.62
	C. odorata	407	567	0.2652	974	916	0.11
Late wet	*A. natalitia*	242	404	0.0371	646	464	0.30
	A. nilotica	299	467	0.0771	765	632	0.84
	D. cinerea	261	434	0.0372	695	454	-0.76
	S. myrtina	279	507	0.0235	786	462	-1.00
	C. odorata	388	581	0.2089	969	896	0.04
	P<	0.001	0.018	0.001	0.001	0.001	0.122
	RMSE	20.018	106.164	0.014	103.657	15.649	1.199
	LSD	19.330	102.510	0.013	100.090	15.111	1.158
Sources of variation effects							
	Se	***	ns	ns	ns	***	ns
	Sp	***	ns	***	***	***	*
	Se x SP	***	ns (P=0.0536)	***	ns	***	ns

[a] Formerly part of Acacia karroo (Coates Palgrave, 2002); [b] invasive non-native species; a_n - the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable; b_n - the proportion of insoluble nutrient which is potentially degradable by micro-organisms; c_n - the degradation rate of fraction b_n per hour; PD_n - potential degradable; ED_n - effective nitrogen degradability; lt_n – lag time; LSD – least significant difference; RMSE - root mean square error; ns (P>0.05), * (P<05); *** (P<0.001).

Table 6.4 Correlation coefficient between chemical composition, and *in sacco* dry matter and nitrogen degradation and estimated parameters

	Chemical constituents						
Parameters	CP	NDF	ADF	ADL	CT	Cell	Hcell
a_{dm}	0.13^{ns}	-0.78***	-0.77***	-0.77***	-0.52*	-0.55*	-0.02 ^{ns}
b_{dm}	0.57*	-0.36 ^{ns}	-0.22 ^{ns}	-0.21 ^{ns}	-0.08 ^{ns}	-0.18 ^{ns}	-0.33 ^{ns}
c_{dm}	0.83***	-0.47 ^{ns}	-0.35 ^{ns}	-0.48 ^{ns}	-0.53*	0.06 ^{ns}	-0.30 ^{ns}
PD_{dm}	0.55*	-0.81***	-0.69**	-0.68**	-0.41 ^{ns}	-0.51 ^{ns}	-0.29 ^{ns}
ED_{dm}	0.76**	-0.86***	-0.70**	-0.74**	-0.62*	-0.41 ^{ns}	-0.38 ^{ns}
lt_{dm}	0.22 ^{ns}	-0.14 ^{ns}	-0.05 ^{ns}	-0.08 ^{ns}	-0.17 ^{ns}	0.04 ^{ns}	-0.22 ^{ns}
a_n	0.65**	-0.66**	-0.59*	-0.65**	-0.56*	-0.27 ^{ns}	-0.17 ^{ns}
b_n	0.22 ^{ns}	-0.31 ^{ns}	-0.12 ^{ns}	-0.13 ^{ns}	0.01 ^{ns}	-0.07 ^{ns}	-0.46 ^{ns}
c_n	0.84***	-0.48 ^{ns}	-0.36 ^{ns}	-0.49 ^{ns}	-0.52*	0.04 ^{ns}	-0.29 ^{ns}
PD_n	0.56*	-0.63*	-0.45 ^{ns}	-0.50 ^{ns}	-0.34 ^{ns}	-0.22 ^{ns}	-0.44 ^{ns}
ED_n	0.82***	-0.74**	-0.58 *	-0.64*	-0.55 *	-0.28 ^{ns}	-0.39 ^{ns}
lt_n	0.05 ^{ns}	-0.65**	-0.58*	-0.52*	-0.51*	-0.57*	-0.17 ^{ns}

CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin; CT - condensed tannin; Cell - cellulose; Hcell - hemicellulose; a_{dm} - the soluble fraction of dry matter which is rapidly washed out of the bags and is assumed to be completely degradable; b_{dm} - the proportion of insoluble nutrient which is potentially degradable by micro-organisms; c_{dm} - the degradation rate of fraction b_{dm} per hour; PD_{dm} – the potential degradability; ED_{dm} - effective dry matter degradability; lt_{dm} – lag time of dry matter; a_n - the soluble nitrogen fraction which is rapidly washed out of the bags and is assumed to be completely degradable; b_n - the proportion of insoluble nitrogen which is potentially degradable by micro-organisms; c_n - the degradation rate of b_n per hour; PD_n - potential degradable; ED_n - effective nitrogen degradability; lt_n – lag time of nitrogen; ^{ns}, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.

6.4 Discussion

Seasonality was hypothesized to affect rumen degradability due to variation in forage quality among seasons. The results supported the hypothesis. The *in sacco* degradability (a_{dm}, PD_{dm} and ED_{dm}) of browse species was lower during the wet season than the dry season in agreement with a previous finding (Camacho *et al.*, 2010). This variation in DM degradability can be attributed to the effects of CT on accessible N, which can decrease ammonia concentrations and microbial growth in the rumen (Salem *et al.*, 2007). Van Soest (1994) suggested that lignin and its cross-linkage to hemicellulose, polysaccharides and proteins could also depress digestibility. High a_n and ED_n of browse species during the early wet season is partly in agreement with Ramírez-Orduña *et al.* (2003) who reported high ED_n of browse plants during autumn and winter at Baja California Sur, Mexico. There may be variation in climate factors between the locations of two studies. Ramírez *et al.* (2000b) suggest that plants may produce new foliage with highly soluble CP due to warm temperatures and wet climate that arise sometime at the end of winter at Northeastern Mexico.

Results showed different effects of the interaction between season and browse species on all dry matter degradability parameters, a_n, c_n and ED_n may be due to variations of chemical composition around the year principally crude protein, fibre fractions and anti-nutritional components. Variations in chemical composition across seasons have been reported by others (Yayneshet *et al.*, 2009; Basha *et al.*, 2012). Based on results of potential and effective degradability in the three seasons and among species, *C. odorata* was greater in quality compared to other browse species. Nevertheless, *C. odorata* is not acceptable to goats that may be attributed to the presence of alkaloids (Prasad *et al.*, 2005).

Rumen degradability was hypothesized to be varying among plant species due to their variation in chemical composition. The results supported the hypothesis. Consistent with our results, Melaku *et al.* (2003) and Anele *et al.* (2009) reported significant variations in DM and CP degradation parameters of multipurpose trees. The DM and CP potential degradability in the current study overlapped the range of 720 – 914 and 546 – 949 g/kg, respectively, reported by Melaku *et al.* (2003). The current study had values that are higher than the range of 362 - 673 for the PD_{dm} reported by Anele *et al.* (2009). Based on potential and effective degradation of both DM and CP, the plant species followed this order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. These differences in the degradation may be associated to the structural and non-structural protein and carbohydrate fractions (Whetton *et al.*, 1997). Previous reports suggested that the variation in the degradation parameters of the browse species may be due to the variation in chemical composition (Khazaal *et al.*, 1993a; Nsahlai *et al.*, 1994; Tolera *et al.,* 1997; Larbi *et al.*, 1998; Kamalak, 2006). Furthermore, the variations in chemical composition between seasons or among plant species have been reported (Nsahlai *et al.*, 1994; Ndlovu & Nherera, 1997; Apori *et al.*, 1998; Larbi *et al.*, 1998; Scogings *et al.*, 2004; Basha *et al.*, 2009) which indicate the variation in the degradation material of the browse species. These variations in PD_{dm} and PD_n in the rumen have been reported as a result of variations in NDF, ADF, lignin (Van Soest, 1994; Yan & Agnew, 2004) and tannins (Kamalak, 2006) or due to other factors such as ash (Benjamin *et al.*, 1995) or maturity (Khazaal *et al.*, 1993a; Kamalak, 2006). Moreover, PD_{dm} and PD_n were negatively correlated with NDF, ADF and CT (Kamalak, 2006). Acid detergent fibre (ADF) and tannins were negatively correlated with PD_{dm} (Vadiveloo & Fadel, 1992; Khazaal *et al.*, 1993b). With regards to *C. odorata*, there has not been any previous report on the PD_{dm} and PD_n.

The soluble fraction of DM (a_{dm}) and CP (a_n) varied within and among plant species, the highest a_{dm} in *A. nilotica* during the three seasons while the highest a_n values recorded in *C. odorata* in the dry and the late wet seasons and *A. nilotica* in the early wet season. Comparable to the others, these differences among these species may be because of variations in the carbohydrates in term of structure and content. The a_{dm} and a_n were negatively correlated with ADF, NDL and CT of browse species (Ramírez *et al.*, 2000a). Tolera *et al.* (1997) and Melaku *et al.* (2003) also reported that a_{dm} and NDF were negatively correlated, and agree with the negative correlation in this study between a_{dm} and a_n, and fibre fractions and CT. In addition, these results are consistent with the lowest values of a_{dm} and a_n in *A. natalitia* in the late wet season and in the two wet seasons, respectively, and *D. cinerea* in the late wet season and in the dry season, respectively. These species had higher fibre fractions in these seasons (Chapter 4).

The greatest value of the slowly degradable fraction of DM (b_{dm}) and CP (b_n) in *A. natalitia* in the dry season and *C. odorata* during the two wet seasons and the lowest values of b_{dm} and b_n recorded in *D. cinerea* in the three seasons and in the early wet season, respectively. These parameters were not related to any measured chemicals in this study except b_{dm} was positively correlated to CP. On the other hand, Ramírez *et al.* (2000a) reported that the slowly degradable fraction of plant cell wall was limited by ADL and tannins and by other factors not measured in the current study such as organic matter, ash and insoluble ash. Many studies reported that the extent of degradation of DM or CP was negatively correlated with NDF, ADF, ADL and CT (Siaw *et al.*, 1993; Bonsi *et al.*, 1995; Melaku *et al.*, 2003).

Markedly higher degradation rate of DM (c_{dm}) and protein (c_n) in *C. odorata* in the three seasons and the slowest rates (c_{dm} and c_n) observed with *S. mytrina* in the three seasons, respectively, reflect differences in chemical composition between the plant species. For instance, *C. odorata* had high CP and low CT contents in the three seasons whilst *S. mytrina* had low CP and high CT (Chapter 4). The result show c_{dm} and c_n are positively correlated with CP but negatively correlated with CT (Table 6.4) and is consistent with findings of Tolera *et al.* (1997) and Kamalak (2006). Larbi *et al.* (1998) reported that the rate of degradation of protein (c_n) was negatively correlated to NDF and ADF concentrations. Melaku *et al.* (2003) found negative relationship between c_n and CT, and

between c_{dm} and neutral detergent fibre bound nitrogen (NDF-N) and ADL, and positive relationship between c_n and NDF-N.

The effective degradability of DM (ED_{dm}) and CP (ED_n) were positively correlated with CP, but negatively related with fibre fractions and CT. This is in agreement with results of previous studies (Larbi *et al.*, 1998; Kamalak, 2006) reporting that ED_{dm} and ED_n were negatively correlated with NDF and ADF concentrations and ED_{dm} was positive correlation to CP concentration. Melaku *et al.* (2003) found negative relationship between ED_n and ADL. The differences in ED_{dm} and ED_n may be attributed to structural and non-structural CP and carbohydrate fractions, which affect protein solubility and bio-availability (Whetton *et al.*, 1997).

6.5 Conclusions

A significant variation in *in sacco* degradability parameters were reported among seasons and among different browse species harvested from sub-humid subtropical savannah of KwaZulu-Natal Province, South Africa, during the dry, the early, and the late wet seasons. These variations are more related with fibre fractions than with tannins content. Fibre concentration appears to be the main factor limiting the *in sacco* degradability. It is suggested that the dilution rate and other factors in the rumen may limit the tannin effects on degradability. Based on potential and effective degradability, the plant species can be placed in the following decreasing order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. Consequently, *C. odorata* is the best supplementary protein source like high-quality leguminous forages. Nevertheless, the *in sacco* results showed that *C. odorata* leaves may have little or no anti-fermentation compounds; however the reluctance of livestock to consume *C. odorata* must be for other reasons. Further studies would need to investigate these reasons and establish new methods for promoting the intake rate of *C. odorata*.

Chapter 7

Relationships between selection index by goats and plant physical characteristics, chemical composition, and digestion parameters of browse species in sub-humid subtropical savannah

Abstract

Diet selection is the key to improving natural pasture management, and leads to better understanding of plant-herbivores interactions. It was hypothesized that the plant characteristics (spinescence, leaf phenology), chemical composition, *in vitro* gas production, *in vitro* degradability or *in sacco* degradability were related to diet selection of browse species. Principal component analysis (PCA) was applied to seek patterns of parameters among themselves and selection index. Regression relationships were estblished between selection index, and chemical compositions (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), condensed tannins (CT), crude protein (CP)), plant characteristics (spinescence and phenology) and the important digestion parameters which were the maximum gas production (GP), the rate of the gas (C), true degradability (TrDeg), degradation efficiency factor (DEF), short chain fatty acids (SCFA), effective degradability of dry matter (ED_{dm}) or nitrogen (ED_n). The PCA grouped the parameters into different groups: the first group included GP, SCFA, CP, C, phen, ED_{dm} and ED_n which were related to each other and related positively to diet selection index (sindex). The second group included the group of the fibres (NDF, ADF and ADL), CT, TrDeg and DEF which had relationships between themselves and were related negatively to sindex. The last group included the sindex and spinescence which were strongly related together. From the PCA results, the addition of PEG improved the relationship between CT and TrDeg, and sindex. Based on regression results, the digestion parameters were poorly related to selection index of goats. The spinescence (spn), phenology (phen), CT, NDF and CP accounted for 86% of the variation in selection index (*y)* = -5.91 - 0.01CT - 0.002NDF + 0.02CP + 6.18spn + 2.43phen; (R^2=0.86; n=20; RMSE=0.406; P<0.001; for phen, 1 = evergreen, 0 = deciduous; for spn, 1 = spinescent, 0 = spineless). It was concluded that *in vitro* gas production, *in vitro* degradability and *in sacco* degradability were poor predictors of selection index of browse species used by

goats. Spinescence, leaf phenology, condensed tannins, CP and NDF were useful predictors of diet selection index.

Key words: effective degradability, polyethylene glycol, microbial yield, portioning factor

7.1 Introduction

Most shrub and browse species are useful livestock fodder, which are the main or even the only source of protein and energy for ruminants in the arid and semi-arid areas. Recently, the value of browse species in the humid tropics has gradually developed due to deficiency in other appropriate feed resources, their high protein content and their use as supplementary feed for ruminants especially in dry seasons. However, the presence of anti-nutritional factors such as tannins in the leaves of browse species limits their utilization (Tolera *et al*., 1997). Therefore, variations in preference for browse species is negatively associated with condensed tannin levels (Kaitho *et al*., 1997), NDF (Mkhize *et al*., 2011) and other chemicals. Mkhize *et al*. (2011) observed a positive relationship between bite size and condensed tannins. Thus, predicting diet selection may allow improving natural pasture management, and leads to better understanding of plant-herbivores interactions.

Physical defence, such as thorns, have been reported to influence diet selection by mammalian herbivores (Provenza & Balph, 1990; Illius *et al*., 1999). These physical features may limit leaf accessibility and intake rates resulting in lower preference of species that have these traits by browsers (Haschick & Kerley, 1997b; Dziba *et al*., 2003a). Sebata and Ndlovu (2010) reported that thorn density influenced the browse instantaneous intake rate through both bite size and bite rate. Cooper (1982) showed that hooked thorns are more effective than straight thorns. Wilson and Kerley (2003b) reported that spinescence affects foraging efficiency of goats. Therefore, the expectation is that spinescence might contribute negatively to diet selection. However, contrary to expectation, Basha *et al*. (2012) showed that spinescent species were selected more than non-spinescent species across the seasons (dry, early wet and late wet) in Zululand Thornveld, South Africa. Shipley *et al*. (1998) found that diet selection by moose is related to plant morphology. Because its leaves are low in photosynthetic rates, slow in growth and function for 2-5 yr (Bliss, 2000), the expectation is that evergreen species may contribute negatively to diet selection and deciduous species positively to diet selection.

This expectation is supported by some previous reports that animals preferred deciduous woody species more than evergreen species (Shipley *et al.*, 1998; Basha *et al.*, 2012).

Chemical composition may explain diet selection, since plant leaves have cell wall (slowly digestible) and cell contents such as proteins that ferment rapidly and may completely be digestible (Bodmer, 1990; Gordon & Illius, 1994). Browse generally contain more indigestible fibers such as lignin, which makes fibre-rich cell walls more difficult and energy-expensive to breakdown (bite and chew) (Choong *et al.*, 1992; Robbins, 1993). Therefore, fibre concentration would be expected to reduce diet selection. However, since proteins are completely digestible, they are expected to positively contribute to diet selection. But, utilization of browse proteins by herbivores may be limited by occurrence of condensed tannins through the formation of tannin-protein binding complexes (Robbins *et al.*, 1991). Thus, our expectation is that condensed tannin could contribute negatively to diet selection. Ørskov and Reid (1989) showed that chemical composition was an imprecise predictor of animal performance via the rate of nutrient and energy intake, which is determined by diet selection. Nevertheless, the information about the relationships between selection of browse species and chemical composition are limited, particularly in sub-humid subtropical areas.

Understanding the basis for diet selection by mammalian herbivores is complex (Shipley, 1999). Their digestive system allows them to extract nutrients from a wide range of vegetation. The vegetation influences post-ingestive effects (Provenza & Villalba, 2006) which may affect diet selection. The post-ingestive effect is based on feedback from the nutrients and toxins of plant material. From this view, the expectation is that diet selection may be affected by the degradability level (post-ingestive).

The objective of the current study was to assess the possibility of predicting diet selection of goats from plant characteristics (spinescence and leaf phenology), chemical composition, rumen degradability or *in vitro* gas production. It was hypothesised that (i) spinesence negatively affect diet selection as long as spinesence has been recognised to affect the foraging behaviour of mammalian herbivores (Papachristou *et al.*, 2003), (ii) phenology is related to diet selection provided that deciduous species are more preferred than evergreen ones (Papachristou & Nastis, 1996; Shipley *et al.*, 1998), (iii) plant chemical composition such as CP, NDF and condensed tannins are negatively related to

diet selection since the chemical composition have been reported to influence diet selection by herbivores (Illius *et al*., 1999; Dziba *et al*., 2003a) and (iv) nutritive value (*in vitro* and *in sacco* degradability) could predict diet selection since the degradability links to the feed palatability and rumen retention time (Provenza & Villalba 2006).

7.2 Methods

7.2.1 Data

Data from Chapters 3, 4, 5 and 6 were used in this chapter. Selection index (sindex), crude protein (CP), neutral detergent factor (NDF), acid detergent factor (ADF), acid detergent lignin (ADL) and condensed tannins (CT) were reported in Chapter 3. For *in vitro* and *in sacco*, the importance parameters which could reflected significant information about nutritive value of the feed were used. Gas production parameters without and with PEG (Chapter 4) included maximum gas production (GP) and gas production rate (C). The true degradability (TrDeg), the total of short chain fatty acids (SCFA) and degradation efficiency factor (DEF) without and with PEG were obtained from Chapter 5. In Chapter 6, *in sacco* degradability was the effective degradability for dry matter (ED_{dm}) and the effective nitrogen degradability (ED_n). The *in sacco* parameters were excluded from the data set with PEG.

7.2.2 Principal components analysis (PCA)

Principal components analysis (PCA) was used for an initial approximation of the relationship between sindex and others parameters using SAS program. Stepwise multiple regression was then used to test the relationship between selection index and variables selected from the PCA, namely, spinescence (1 = spinescent; 0= spineless), leaf phenology (1 = evergreen; 0 = deciduous), chemical composition (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), condensed tannins (CT), crude protein (CP)), *in vitro* gas production, *in vitro* degradability and *in sacco* DM or N degradability parameters of plant species. The probability of a variable entering the model was set at 0.15 and the removal of early entry variables was allowed in the model if they were made redundant by new variables.

7.3 Results

7.3.1 Pattern of relationships by PCA

The patterns of sindex, chemical compositions, plant characteristics (spinescence and phenology), GP, C, TrDeg, DEF, SCFA, ED_{dm} and ED_n (without PEG) obtained by PCA were shown in Figure 7.1. The PCA generated two factors; the variance explained by factor 1 and factor 2 were 80% and 28%, respectively. The patterns of the parameters showed different relationships among these parameters and sindex. The following parameters: GP, SCFA, CP, C, phen, ED_{dm} and ED_n had strong relationship with each other and were positively related to sindex. The fibres (NDF, ADF and ADL), CT, TrDeg and DEF had strong relationships with each other but were negatively related to the sindex. The spine variable showed a strong and positive relation to sindex.

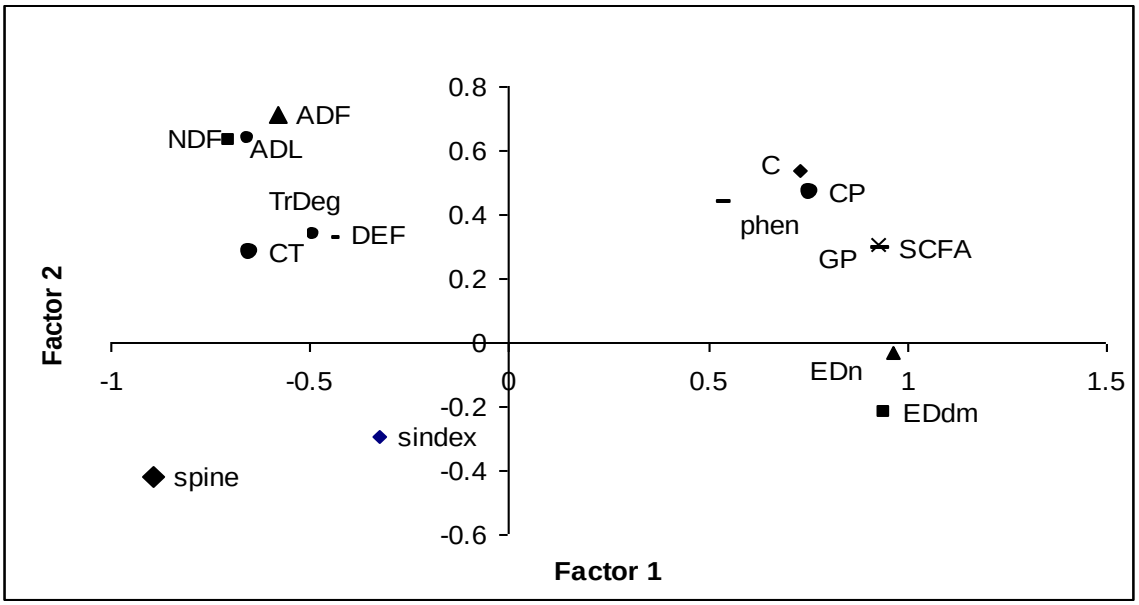

Figure 7.1 Plot of factor pattern for Factor 1 and Factor 2 (without PEG) of PCA.

Sindex – diet selection index; spine – spinescence; phen – phenology; CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; CT – condensed tannin; GP=maximum gas production; C = rate of gas production; TrDeg – true degradability; DEF - degradation efficiency factor; SCFA – total of short chain fatty acids; ED_{dm} - effective dry matter degradability; ED_n - effective nitrogen degradability.

The patterns of sindex, chemical compositions, plant characteristics (spinescence and phenology), GP, C, TrDeg, DEF and SCFA) with PEG obtained by PCA are presented in Figure 7.2. Factor 1 explained 60% and factor 2 explained 38% of the variation. PEG improved and shifted the relation between CT and TrDeg, and sindex. The *in vitro* and *in sacco* parameters had no relationship with diet selection index.

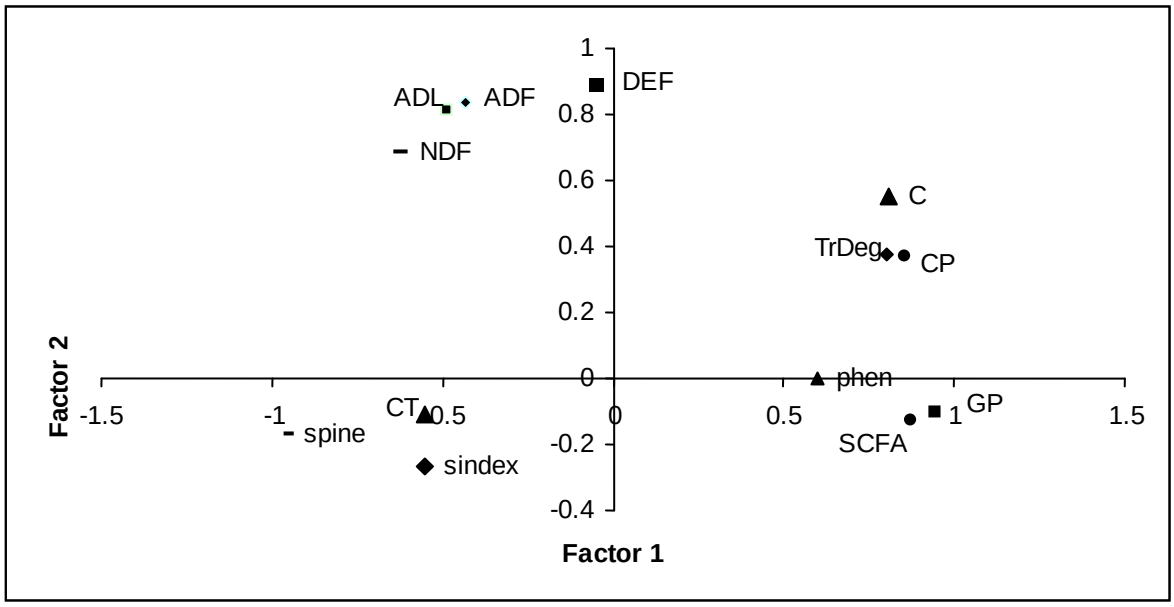

Figure 7.2 Plot of factor pattern for Factor 1 and factor 2 (with PEG) of PCA.

Sindex – diet selection index; spine – spinescence; phen – phenology; CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; CT – condensed tannin; GP=maximum gas production; C = rate of gas production; TrDeg – true degradability; DEF - degradation efficiency factor; SCFA – total of short chain fatty acids.

7.3.2 Pattern of relationships by multiple regression model

The multiple regression model of the relationship between selection index and spinescence (Spn), leaf phenology (Phen), condensed tannins (CT), NDF and CP accounted for 86% of the variation in selection index (y). The relationship was:

$$y = -5.91 - 0.01CT - 0.002NDF + 0.02CP + 6.18Spn + 2.43Phen \quad \text{(equation 7.1)}$$

(R^2=0.86; n=20; RMSE=0.406; P<0.001)

(for phen, 1 = evergreen, 0 = deciduous; for spn 1 = spinescent, 0 = spineless)

Spinescence, leaf phenology and CP had positive contributions to selection index while both CT and NDF had negative contributions to selection index. All other variables had poor relationship with sindex.

7.4 Discussion

The positive relationships among the group of GP, SCFA, CP, C, phen, ED_{dm} and ED_n indicated on PCA (Figure 7.1) may be supported given the effect of CP concentration on *in sacco* degradability via it effects on the population of rumen microbes and its enzymes, since protein is a main requirement for the rumen's micro-organisms (Carro *et al*, 1991). Kaitho *et al*. (1998a) reported positive relationships between effective degradability of the dry matter and potential gas production of browse species. Many previous studies (Khazaal *et al*., 1993b; Sileshi *et al*., 1996; Tuah *et al*., 1996; Apori *et al*., 1998; Kamalak *et al*., 2004; Gasmi-Boubaker *et al*., 2005) reported that *in sacco* dry matter degradability and gas production were positively correlated. Moreover, gas production signals the generation of SCFA and microbial mass (Getachew *et al*., 1998), and hence supports the relationship between DM degradability and SCFA. Melaku *et al*. (2003) found relationship for *in sacco* ED_{dm} with *in vitro* SCFA.

The relationships among NDF, ADF, ADL, CT, TrDeg and DEF were similar to the relation between the cell wall fraction and browse digestibility (Wilson, 1977; Mertens, 1993). Moreover, Reed *et al.* (1990) reported that tannins are present in the fibres (NDF and ADF) and are bound to the cell wall and protein and appear to be responsible for decreasing digestibility. It has been reported that the decrease in digestibility of dietary fibres is due to the anti-nutritional factors present in the feed, which affect fibrolytic micro-organisms (Reed, 1995; Ngwa *et al*., 2001).

The negative relationships between the groups obtained by PCA agree with the findings of Kabuga and Darko (1993) who recorded linear decrease of DM degradability with increased fibre content. The negative relationship between the *in sacco* DM degradability, and NDF and ADL were reported (Minson, 1982; Nsahlai *et al*., 1994; 1995; Kaitho *et al*.,

1998a) while Kamalak *et al*. (2004) reported a negative relationship between *in sacco* DM degradability and NDF.

Generally, the interference of tannin might influence the relationship among the digestion parameters of browse species. The relationship among these parameters improved when PEG was added to browse samples, since there is a strong negative relationship between gas production and condensed tannin (CT) contents. Previous studies reported that tannin-containing feeds with PEG led to increase in gas production (Makkar *et al*., 1995a; Getachew *et al*., 2001; 2002; Seresinhe & Iben, 2003; Mauricio *et al*., 2009). However, in another study the addition of PEG improved the relationship between CT and TrDeg, and sindex (Figure 7.2). This could be attributed to a depressive tannin effect due to PEG, and an increase in the available nutrients for rumen microbes (Makkar *et al*. 1995a; Getachew *et al*., 2000a; Rubanza *et al*., 2005; Nahand *et al*., 2010). Moreover, the increasing in gas production of tannin-rich feed by adding PEG was reported, with no effect on true DM degradability (Osuga *et al*., 2008).

Because the main focus of this chapter was on the relationship between sindex and the others parameters, the discussion is restricted to the model reported in the results which established relationship between sindex and five variables (spinescence, leaf phenology, CP, CT and NDF). The prediction of diet selection by plant characteristics is more useful since the diet selection acts as a key process in plant-herbivore interactions and plant species are important factors that influence the patterns of diet selection (Dziba *et al*., 2003a; Mkhize, 2008; Baraza *et al*., 2009; Basha *et al.,* 2009; 2012). Contrary to expectation (Papachristou *et al*., 2003), spinescent species have a positive contribution to diet selection index while spineless species have no contribution. This may be attributed to the fact that four of the five studied species being spinescent. This is also supported since thorny species such as *Scutia myrtina*, *Acacia natalitia* (Mkhize, 2008) and *Acacia karroo* (Haschick & Kerley, 1997a) are among the preferred species. Hence, some previous studies reported variations in diet selection patterns of spinescent species (Owen-Smith & Cooper, 1987; Mkhize, 2008). In addition, spinesence has been recognised to affect the foraging behaviour of mammalian herbivores (Cooper & Owen-Smith, 1986; Papachristou *et al*., 2003) by limiting animals that forage on spinescent species (Woodward & Coppock, 1995). On the other hand, Gowda (1996) suggested that spinesence influences foraging behaviour more by spine traits (shape, length and sharp) than just the presence or absence

of spines. Such morphological traits do not deter animals from foraging but reduce bite size and bite rate (Cooper & Owen-Smith, 1986). This is consistent with Wilson and Kerley (2003b) who showed a positive relationship between foraging rate and plant morphology. Preference of spinescent species was higher than that of spineless species within and across the seasons (Mkhize, 2008; Basha *et al.,* 2009); this is contrary to Haschick and Kerley (1997b) who showed a lower preference for spinescent plants.

Contrary to expectation, evergreen species have a positive contribution to diet selection while deciduous species have no contribution. Previous studies reported that evergreen and deciduous species were among the more and less preferred species (Dziba *et al*., 2003a; Mkhize, 2008). Mkhize (2008) reported that evergreen species which are broad-leaved were preferred by goats during the dry and late wet seasons more than deciduous species which are fine-leaved. Herbivores could obtain larger bite sizes from broad-leaved species than fine leaved ones (Dziba *et al*., 2003a; Wilson & Kerley, 2003b). However, the deciduous species are more preferred than evergreen ones (Papachristou & Nastis, 1996; Shipley *et al*., 1998). Jefferies *et al*. (1994) added that the evergreen species compared with deciduous plants are less palatable, less digestible, and more toxic to large and small mammals and insects.

Beside physical defence and phenology the differences in chemical composition have been reported to influence diet selection by herbivores (Illius *et al*., 1999; Dziba *et al*., 2003a). The incorporation of CP, NDF and CT in predicting diet selection index made a reasonable sense and contribution. Condensed tannin usually reduces feed intake and digestibility (Ben Salem *et al*., 1999; 2005a). Herbivores are able to balance diet selection by maximizing nutrient intake and minimizing plant secondary compounds (Jansen *et al*., 2007). That fibre (NDF) had a negative contribution to diet selection index agrees with others (Ganqa *et al*., 2005), thus reducing intake and digestion of nutrients (Lu *et al*., 2005). By controlling mastication, fibre limits intake by increasing rumen retention time and maintains normal fermentation in the rumen by introducing saliva (Lu *et al*., 2005). Cooper *et al.* (1988) reported that food selection could be affected negatively by fibre components. Moore and Jung (2001) reported a negative relationship among the fibre fractions and the digestibility of browse.

The CP had a positive contribution to selection index of browse species and agrees with previous studies that reported that selection of food depends upon protein levels (Nyamangara & Ndlovu, 1995; Dziba *et al.*, 2003a; Ganqa *et al.*, 2005). Consequently, it is much better to use more than one variable to relate diet selection. This supports the suggestion of Stensig *et al.* (1994) and Madsen *et al.* (1997) recommending the use of more than one degradation parameter for prediction of feed intake. Moreover, Getachew *et al.* (2004) reported that GP alone was a poor predictor of *in vitro* TrDeg, but insertion of CP, non-fibre carbohydrate, and fat levels in the predictive equations significantly improved the prediction of the *in vitro* TrDeg from GP.

7.5 Conclusions

The use of *in vitro* gas production, *in vitro* degradability and *in sacco* degradability parameters in a multiple regression to predict selection index of browse species by goats showed poor relationship. Diet selection index had a strong relationship with spinescence, leaf phenology, condensed tannins, NDF and crude protein contents.

Chapter 8

General discussion, Conclusions and Recommendations

8.1 General discussion

Currently, sustainable livestock production especially in African savannahs is becoming more of a challenge due to the problem of insufficient forage throughout the year. A great number of shrub and browse species in savannahs are valuable livestock fodders. Feeding browse species to small ruminants is imperative and could constitute a vital strategy in savannahs. Several browse species have been introduced in ruminant feeding programs because they have high nutritive potential and selectivity, principally during dry periods. However, a pattern of selection of these browses by animals needs to be understood in order to optimise animal productivity.

The overall objective of this study was to determine the patterns of diet selection of herbivores on pasture woody species in sub-humid subtropical savannah, South Africa and predict the diet selection using plant characteristics and nutritive value of browse species such as chemical composition, *in vitro* gas production, *in vitro* degradability and *in sacco* degradability.

Based on literature, in subtropical and tropical regions the lack of sufficient year-round feed resources is the most important factor that causes low growth rates and low animal production, especially during the dry season (Makkar & Becker, 1997a). The use of browses as forage for ruminants is gradually becoming important, but the limitation in the use of these plant species is nutritional value (Aganga & Tshwenyane, 2003). Browses have anti-nutritional factors (particularly tannins), that limit their nutritive value (Makkar & Becker, 1998; Aganga & Tshwenyane, 2003). Physical features such as thorns and spines may limit leaf accessibility and intake rates resulting in lower preference of species that have these traits by browsers (Dziba *et al*., 2003a). Additionally, odours associated with particular trees or shrubs have been reported (Leng, 1997). They act as defences of plants against herbivores and other predators.

In this study, Chapters 2 and 3 dealt with diet selection using different methodologies. It was demonstrated that diet selection varied among the seasons. The top five species selected by goats were *Acacia natalitia*, *Acacia nilotica*, *Scutia myrtina*, *Dichrostachys cinerea* and *Chromolaena odorata*. *Scutia myrtina* was the most preferred species during the dry season while *D. cinerea* was the most preferred in the wet season. *Scutia myrtina* is an evergreen species and most of the other species are deciduous in the study area. Consequently, during the dry season the choices of diet are narrow when there is low availability of palatable materials. Spinescent species were generally preferred in all seasons, particularly during the dry and early wet seasons. Deciduous species dominated the diet in the wet seasons particularly during the early wet season. The selection index was used for ranking species according to selectivity in the field and this was instrumental in selecting the five top ranked species for *in vitro* trials presented in Chapter 4 and 5 and *in sacco* trial in Chapter 6.

Chapter 4 dealt with gas production and Chapter 5 dealt with *in vitro* DM degradability. The main results of *in vitro* fermentation include the impacts of season, plant species and their interaction.

Depending on the *in vitro* fermentation information, condensed tannins is the most important factor that affects the nutritive value of browse species by limiting of the browse species utilization by ruminant animals. Tolera *et al*. (1997) pointed out that the occurrence of tannins and other phenolic compound in a great number of shrub and tree leaves restrict their utilization. Reduced palatability, intake rate and digestibility are reasons for the negative effects of tannins on ruminants feed intake (Provenza, 1995). Use of polyethylene glycol (PEG) *in vitro* to negate effect of tannin has been shown by several workers (Makkar *et al*., 1995a; Jones *et al*., 2000; Getachew *et al*., 2001; Kamalak *et al*., 2005; Rubanza *et al*., 2005; Vitti *et al*., 2005; Nahand *et al*., 2010; Sallam *et al*., 2010; Nsahlai *et al*., 2011). The addition of PEG decreased the lt, T1/2, PF due to TrDeg but increased the GP, C, total SCFA and gas kinetics parameters (Table 4.5, 4.7, 5.3 and 5.5). The addition of PEG emphasizes that the inhibitory effect of tannins on rumen microbes was great for tannin-rich feed.

In Chapter 6, plant species had lower *in sacco* DM potential degradability as compared to true degradability *in vitro* (TrDeg). Although a lower degradability *in vivo* as compared to

in vitro is generally expected (Rymer *et al*., 2005), the negative relationships between PD_{dm} or ED_{dm} and fibre fractions (NDF, ADF and ADL) (Table 6.3) indicate that the lower PD_{dm} could be due to the fibre fractions contents.

The predicting of diet selection of browse species using the digestion parameters was poor as indicated by the poor relationship between these parameters and diet selection. However, spinescence, phenology, NDF, CP and CT were predictors of diet selection index (equation 7.1).

8.2 Conclusions

The preference of plant species varied between the seasons. The species which were not preferred in the wet season by goats may become more preferred in the dry season; for instance *S. myrtina* was moderately preferred during the wet season but it was the most preferred during the dry season and the reverse was true for *D. cinerea*, which was highly preferred during the wet season and less preferred during the dry season.

Significant variations in *in vitro* gas production and fermentation kinetics were reported among seasons and among different browse species. These variations were associated with the CP content or tannin content. The levels of condensed tannin concentration limited *in vitro* gas production of tannin-rich browse species. The addition of PEG enhanced the extent of fermentation, especially of tannin-rich browses. It is suggested that PEG should be used as detannification agents in goats foraging this pasture.

Also, *C. odorata* can be used as supplementary protein source. The *in vitro* GP results showed that *C. odorata* leaves may have little or no anti-fermentation compounds; however the reluctance of livestock to consume *C. odorata* is not related to its high protein and must be for other reasons. Further studies would need to investigate these reasons and establish new methods for promoting intake rate of *C. odorata*.

In vitro degradability parameters varied among seasons and among different browse species. The addition of PEG emphasizes the inhibitory effect of tannins on rumen microbial activity. The results suggest that in order to use these browse species efficiently, there is need to reduce the effect of condensed tannins.

The variation in *in sacco* degradability parameters were reported among seasons and among browse species. These variations were more strongly related with the fibre fractions than with tannins content. The levels of fibre concentration appeared to be the main factor limiting *in sacco* degradability parameters. Based on potential and effective degradability, the plant species followed this order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*.

In vitro gas production and digestibility, and *in sacco* degradability parameters had poor relationship with selection index of browse species by goats. However, selection index was strongly and positively related to plant characteristics (spinescence, phenology) and CP; and negatively related to NDF, and CT; all explaining 86% of the variation.

8.3 Recommendations

Goats play a significant role in the livelihood of rural people in the KwaZulu-Natal province in South Africa. Goats do well in tropical regions because of their ability to feed on diverse types of plant species, mainly browses and grasses. It is therefore necessary to, assess and estimate the pasture contents.

It is wise to apply more than one evaluating method when ranking plant species in terms of their significance to animals since a single method can show misleading results. For instance, in this study when plant species were ranked based on selection index, it was obvious that in the wet season *D. cinerea* was the most selected species than *C. odorata* although, which was more available in the field than *D. cinerea*. But when applying *in vitro* gas production or *in sacco* degradability, it became noticeable that *C. odorata* had the highest fermentation parameters than other species. Using selection index alone has some restrictions; e.g. a plant species was selected by the animal but was not recorded in the availability data and consequently selection index was zero. Preference is dependant on plant availability in the environment. It is necessary to develop a method that avoid such restrictions.

The species included in the goat's diet covered a range of physical traits or phenology. Spinescent species were selected more than spineless species, while fine-leaf or deciduous species were selected more than broad-leaf or evergreen species. Characterization of plants

browsed by goats in the study area (Zululand Coastal Forest and Thornveld, South Africa) would donate the understanding and improved management system of their impact on the vegetation they utilise. A further study of goat's impact on the vegetation is necessary to determine vegetation changes that may take place in future for management intentions. Furthermore, veld assessment should be done at regular intervals in order to determine whether retrogression is continuing.

However, the distinctions in selectivity of browse species may change due to the adaptation of goats or huge difference of their nutritional status throughout the year. For example, some browse species that are least preferred during periods when feeds availability is high might be preferred during periods when existing feeds are inadequate. As a result, including a diversity of browse species in natural pastures will guarantee year-round availability of feeds for improved animal performance.

Since *C. odorata* had high CP and low CT and high potential and effective degradability, its low preference by goats might also be associated to some specific metabolites that modulate animal satiety; this needs further research to investigate and establish new methods for promoting the intake rate of *C. odorata*.

Goats in many parts of Africa depend on browse species in the dry season or during years of drought because of the low nutritive value of available grass. *Acacia* species contain tannins. The effect of tannins on the *in vitro* fermentation characteristics of *Acacia* species was established through the use of PEG in the *in vitro* gas production technique. In general, inactivation of tannins resulted in increased gas production and degradability. Nevertheless, inactivation of tannins with PEG is still not used by smallholder farmers for improving the nutritive value of *Acacia* species. Subsequent research should extablish the amount per animal per day and the method of offering (in water, as drench or in feed).

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Appendices

Appendix 1 Study area at Owen Sitole College of Agriculture (OSCA), South Africa.

Appendix 2 Study herd of goats while foraging.

Appendix 3 Condensed Tannins (proanthocyanidins)

Regants:

1. Aqueous acetone, 70%: Mix 700 ml of acetone with 300 mo of water.
2. Butanol-HCl reagent: Mix 950 ml of n-butanol with 50 ml of concentrated HCl (37%).
3. Ferric reagent (2% ferric ammonium sulfate in 2 M HCl): Dilute 16.6 ml of concentrated HCl (37%) to 100 ml with water to make 2 M HCl, dissolve 2.0 g ferric ammonium sulfate in this volume of 2 M HCl. Note: This reagent should be stored in a dark bottle in refregerater.

Determination:

- Weigh, accurate to 0.0001 g, 200 mg of a sample (ground to pass a 100 mesh (150 micron) sieve) in abeaker of 20 ml and add 10 ml of 70% agueous acetone.
- Place he beaker in an ice bath and subject it to ultrasonic treament for 3 minutes (Repeat the ultrasonic tretment three times for a total period of 12 minutes).
- Centrifuge the contents (preferably at 4 °C) for 2o minutes at about 5000 rpm (4370g).
- Collect the supernatant in a glass tube and keep it on ice.
- Make appropriate dilutions of tannin extract with 70% acetone.
- Pipette 1 ml of the tannin extract diluted with 70% acetone into a test tube.
- To the tubes add 6 ml of the butanol-HCl reagent and 2 ml of the ferric reagent .
- Vortex mix the tubes.
- Cover the mouth of the tube with reaper lids and put the tubes in an water bath adjusted at 97 to 100 °C for 60 minutes.
- Cool the tubes and measure the abosorbance at 550 nm. Substract the absorbance of a suitable blank, wich is usually the absorbance of the unheated mixture.
- Content of condensed tannins, as leucocyanidin equivalent. Is calculated by the following formula:

$$\text{Condensed tannins (\% in dry matter)} = \frac{A_{550nm} \times 78.26 \times D}{\text{\% dry matter}}$$

A_{550nm} = Absorbance at 550 nm

78.26 = Accumulative factor taking into account: extinction coefficient of leucocyanidin. Mass of sample (200 mg) and other factors except dilution.

D = Dilution factor.

Appendix 4 Ankom Fibre analysiser apparatus.

Appendix 5 Automated *in vitro* gas production technique.

Appendix 6 GC apparatus.

Appendix 7 Fistulated cow.

Appendix 8 Chemical composition (g/kg DM) of *Eragrostis* hay and Lucerne hay

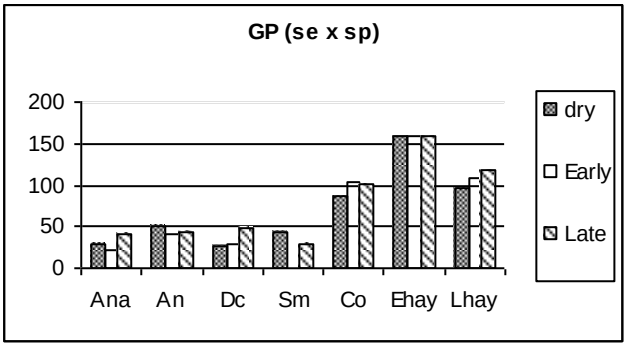
Feed	CP	NDF	ADF	ADL
Eragrostis hay	121	843	383	67.8
Lucerne hay	157	536	327	90.2

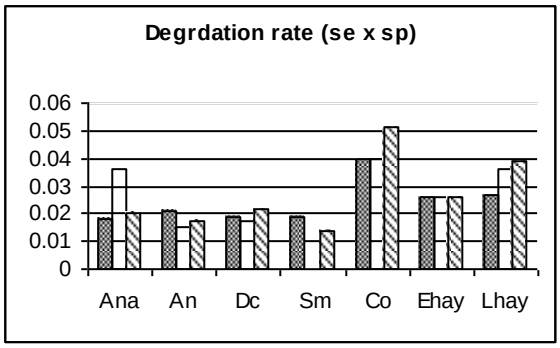
CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin

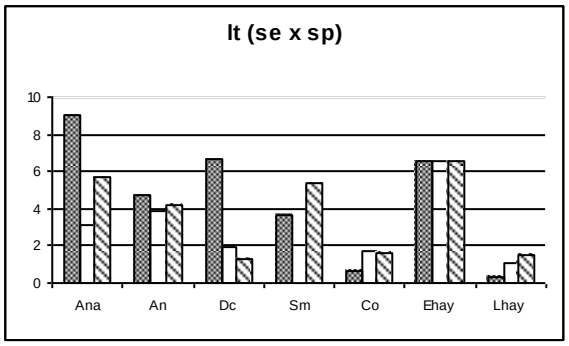
Appendix 9 Gas production kinetics of *Eragrostis* hay and Lucerne hay

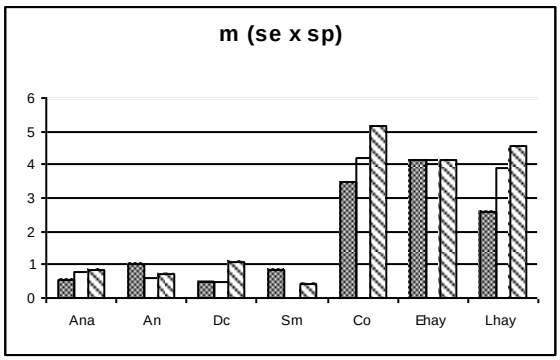
		Eragrostis hay					Lucerne hay				
Season		Dry	Early wet		Late wet		Dry	Early wet		Late wet	
PEG		-	-	+	-	+	-	-	+	-	+
Experiment 1											
Parameters	GP	160	160	-	160	-	96	108	-	117	-
	A	35	34	-	34	-	46	54	-	61	-
	B	125	126	-	126	-	50	55	-	57	-
	C	0.026	0.026	-	0.026	-	0.027	0.036	-	0.039	-
	c1	0.095	0.099	-	0.098	-	0.133	0.129	-	0.135	
	c2	0.025	0.025	-	0.025	-	0.026	0.032	-	0.033	-
	lt	6.6	6.5	-	6.5	-	0.4	1.1	-	1.5	-
	µ	4.2	4.1	-	4.1	-	2.6	3.9	-	4.5	-
	$T_{1/}$	26	26	-	26	-	19	15	-	15	-
Experiment 2											
Parameters	GP	-	167	154	155	167	-	113	124	106	122
	A	-	36	35	34	37	-	55	59	55	57
	B	-	131	120	122	129	-	58	65	51	64
	C	-	0.025	0.027	0.025	0.026	-	0.034	0.033	0.042	0.035
	c1	-	0.097	0.101	0.097	0.096	-	0.124	0.108	0.143	0.120
	c2	-	0.025	0.026	0.025	0.026	-	0.031	0.029	0.036	0.031
	lt	-	6.6	7.7	6.2	8.0	-	0.9	1.4	1.1	1.4
	µ	-	4.2	4.1	3.9	4.4	-	3.9	4.1	4.4	4.3
	$T_{1/}$	-	26	26	26	27	-	16	17	13	16

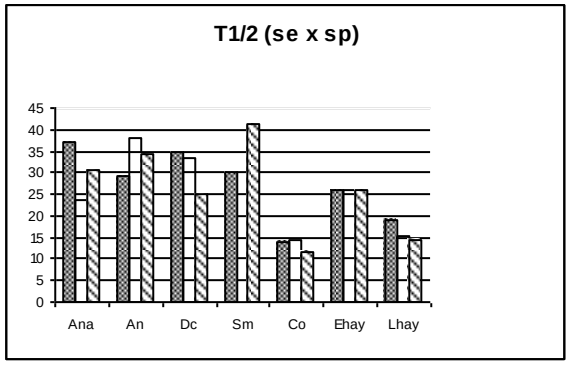
GP=maximum gas production; A and B=gas volume (ml) from fast (cell content) and slowly (cell wall) degradable fractions, respectively; C=rate of gas production (h^{-1}); c_1 and c_2= degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively; lt=lag time; µ=maximum rate of gas production at the point of inflection of the gas curve; $T_{1/2}$ =half time to the maximum gas volume.











Appendix 10 Interactions between seasons and plant species on gas production parameters of browse species harvested from sub-humid subtropical savannah, South Africa. Ana - *Acacia natalitia*; An - *Acacia nilotica*; Dc - *Dichrostachys cinerea*; Sm - *Scutia myrtina*; Co - *Chromolaena odorata*; Ehay *is Eragrostis* hay; Lhay isLucerne hay; GP - maximum gas production; lt - lag time; μ - maximum rate of gas production at the point of inflection of the gas curve; $T_{1/2}$ - half time to the maximum gas volume.

Appendix 11 *In vitro* degradability and total of short chain fatty acids of *Eragrostis* hay and Lucerne hay

	Eragrostis hay					Lucerne hay				
Season	Dry	Early wet		Late wet		Dry	Early wet		Late wet	
PEG	-	-	+	-	+	-	-	+	-	+
Experiment 1										
Parameters										
pH	6.64	6.62	-	6.61	-	6.79	6.75	-	6.74	-
ApDeg	445	420	-	411	-	512	383	-	425	-
TrDeg	743	702	-	702	-	869	673	-	706	-
MY	297	410	-	317	-	357	446	-	329	-
SCFA	52.6	51.9	-	51.8	-	65.6	68.4	-	63.0	-
PF	4.65	4.39	-	4.39	-	9.04	6.21	-	6.03	-
DEF	0.36	0.34	-	0.34	-	0.95	0.82	-	0.83	-
Experiment 2										
Parameters										
pH	-	6.62	6.64	6.61	6.72	-	6.75	6.76	6.74	6.78
ApDeg	-	419	400	383	464	-	411	433	425	430
TrDeg	-	702	715	701	733	-	673	761	706	733
MY	-	410	314	317	303	-	446	328	329	303
SCFA	-	51.9	51.2	51.8	51.5	-	68.4	67.7	63.0	68.2
PF	-	4.22	4.73	4.54	4.56	-	5.97	6.17	6.74	6.05
DEF	-	0.32	0.38	0.35	0.34	-	0.77	074	1.05	0.78

ApDeg - the apparent degradability (g kg^{-1} DM); TrDeg – true degradability (g kg^{-1} DM); MY – microbial yield (g kg^{-1} DM); SCFA – total of short chain fatty acids (mmol L^{-1}); PF – portioning factor; DEF=degradation efficiency factor